International Symposium on Epidermolysis Bullosa

Chapel Hill, North Carolina, April 25–26, 1994

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Jo-David Fine, M.D., M.P.H. (Committee chairman), University of North Carolina at Chapel Hill, Chapel Hill, NC
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Karen Holbrook, Ph.D., University of Florida, Gainesville, FL
Jouni Uitto, M.D., Ph.D., Jefferson Medical College, Philadelphia, PA

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EPIDERMOLYSIS BULLOSA: EPIDEMIOLOGICAL, ULTRASTRUCTURAL AND IMMUNOPATHOLOGICAL STUDY.

C.Angelo, M.Paradisi, A.Morricone, C.Mazzanti, P.Puddu, A.Onetti Muda, T.Faraggiana*, R.Cavalieri. I.D.I., IRCCS–ROMA; *Dipartimento di Medicina Sperimentale – Univ. La Sapienza – ROMA*

During the years 1987–1992 we examined in our Institution 27 family groups which were affected by Ereditary EB; a total of 61 patients was then selected, and a specific diagnosis was given on the basis of clinical, histological and/or ultrastructural examination.

Skin biopsies were taken from selected cases to investigate the feasibility and reproducibility of non conventional fixation and embedding procedures to study the distribution of specific antigens, such as Collagen types III, IV and VII, and Laminin.

Skin biopsies were obtained from cases of herpetiform EBS and of recessive EBD. Tissue samples were rapidly frozen and freeze–substituted to achieve an optimal dehydration without treatment with chemical solvents and embedded in paraffin or in resin. All sections were also observed with a Confocal laser scanning microscope (CLSM) for resolution enhancement of the image, three–dimensional image reconstruction and image analysis procedures.

Our results showed that cryofixation and freeze–substitution followed by paraffin or resin embedding are the techniques of choice to obtain excellent antigen reactivity in conjunction with detailed structure and ultrastructure. Confocal microscopy allowed us to gain optical sections free of out–of–focus blur from up to 100 microns thick tissue blocks stained with fluorescent or reflecting probes, and therefore obtaining:

1. a 50–fold enhanced resolution in the specimen plane
2. a three dimensional reconstruction of a set of optical sections taken at different focal planes
3. an animated sequence of the 3D image to reveal latent features of the specimen

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THE 150 kDa CHAIN OF NICEIN/KALININ IS A TRUNCATED ISOFORM OF LAMININ α1 CHAIN DISTINCT FROM THE HEAVY CHAIN OF k-LAMININ.

C. Baudoin, C. Miquel, D. Aberdam, J.-P. Ortonne, G. Meneguzzi. INSERM U385, Faculté de Médecine, Université de Nice, France.

We have isolated and characterized the full-length cDNAs for the 150 kDa subunit (α3 chain) of nicein/kalinin (laminin-5). The 5148 nt sequence comprises an open reading frame of 4668 bp encoding a protein of 1556 amino-acids (173,211 kDa). Four distinct regions homologous to domains of human laminin α1 chain were identified: -1) a rod-like region, 20.6% homologous to the α-helical domain I-II, containing a KVAV and a RGD sequence at the carboxyl end; -2) an EGF-like region, with cysteine-rich repeats organized in three blocks of ~60 amino acids, 28% homologous to residues 1321-1543 (domain III); -3) a short N-terminal globular region displaying a 23% homology to the domain VI; -4) a G domain, 22% homologous to residues 2313-2808, composed of 3 sub-domains. A polyclonal antibody (SE85) elicited against a bacterial fusion protein, corresponding to the C-terminal domain of laminin α3 chain, specifically reacted with purified laminin-5 and labeled epidermal basal membranes. It also induced detachment of adherent keratinocyte cultures without affecting fibroblasts. SE85 antibody did not label the skin basement membrane of a Herlitz's junctional epidermolysis bullosa (H-JEB) patient. Since antibody BM165 to the G domain of laminin-5 and k-laminin (laminin-6) reacted with this H-JEB skin, we concluded that the α chains of the two isolaminins present distinct antigenic determinants. Northern blot analysis of RNA extracted from keratinocytes of the H-JEB patient with laminin α3 chain cDNA probes did not detect expression of the corresponding mRNAs. We therefore, suggest that laminin-5 and laminin-6 comprise distinct α chain isoforms.

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PREMATURE TERMINATION CODONS ON BOTH ALLELES OF THE TYPE VII COLLAGEN GENE (COL7A1) IN THREE JAPANESE BROTHERS WITH RECESSIVE DYSTROPHIC EPIDERMOLYSIS BULLOSA.

Angela M. Christiano, Yasushi Suga*, Alain Hovnanian, Daniel S. Greenspan^, Hideoki Ogawa* and Jouni Uitto.** Jefferson Medical College, Philadelphia, PA; *Juntendo Universitiy, Tokyo, Japan; INSERM, Creteil, France; and ^University of Wisconsin, Madison, WI.

We have recently demonstrated premature termination codons on both COL7A1 alleles of two Hallopeau-Siemens RDEB patients, and on one allele of fourteen others. In this study, we screened for mutations in COL7A1 using PCR-amplified genomic DNA followed by heteroduplex analysis in a Japanese family with three brothers affected with HS-RDEB. In a PCR product spanning exons 7-9 of COL7A1, we identified a C-to-A transversion on one allele of the clinically unaffected mother and the three brothers with RDEB. This mutation converted a tyrosine residue (TAC) to a stop codon (TAA) at bp 933 in exon 7, and is designated Y311X. The inheritance of this mutation was confirmed in the family using a newly created restriction site for DdeI (CTNAG). In a PCR product spanning exons 69-71 of COL7A1, we identified a 1 bp deletion in one allele of the clinically unaffected father and the three brothers with RDEB. This mutation, 5819delC, results in a frameshift and premature termination codon (TGA) 64 amino acids downstream from the deletion, in exon 73 of COL7A1. The inheritance of this mutation in the family was verified using a newly created restriction site for AciI (GCGG), and a deleted site for MspI (CCGG). In this family, the clinical phenotype results from the brothers being compound heterozygotes for two mutations resulting premature termination codons on both alleles of COL7A1. The consequence of these mutations is the absence of anchoring fibrils in the skin of these three patients, due to the lack of any full-length type VII collagen polypeptides.

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DYSTROPHIC EPIDERMOLYSIS BULLOSA COMPLICATED BY SQUAMOUS CELL CARCINOMA: WORTH A SECOND LOOK.

MGS Dunnill, GM Levene, PH McKee, BJ Mayou, RAJ Eady. Institute of Dermatology and Departments of Histopathology and Plastic Surgery, St Thomas' Hospital, London, UK.

Squamous cell carcinoma is a well-recognised complication of recessive dystrophic epidermolysis bullosa (EB) and may be associated with other forms of EB. EB pruriginosa is a clinical subtype of EB characterised by the presense of lichenoid and prurigo-like lesions associated with blistering and scarring affecting mainly the lower legs but also other sites[1]. We report a case of EB pruriginosa which has been complicated by two squamous cell carcinomas (SCCs). Our patient is a 32 year old woman who was born with loss of skin over her left lower leg which was encircled by the umbilical cord. The defect was treated with a skin graft. During infancy she suffered from blistering and scarring induced by minor trauma, mainly localised to lower legs and forearms. These areas have always been very itchy. Over her teenage years she developed violaceous linear scarring with a lichenoid or nodular prurigo-like appearance. She is the only child of two normal, unrelated parents. When aged 30 she noticed a hard crusted area on the right shin. A biopsy of this area revealed only pseudo-epitheliomatous hyperplasia. Despite treatment with antibiotics and occlusion, the lesion continued to grow and was excised. Eight months later a similar lesion developed on the left shin and again an initial biopsy showed no evidence of malignancy. This was excised on clinical suspicion. Both excised lesions showed histological changes of well-differentiated SCC with a chronic inflammatory cell reaction and extensive scarring in the underlying dermis.

This case illustrates the risk of SCC complicating one of the milder forms of DEB, and the importance of a high index of suspicion of malignancy even in apparently benign lesions.

1. McGrath JA, Schofield OMV, Mayou BJ, McKee PH, Eady RAJ. Epidermolysis bullosa complicated by squamous cell carcinoma: report of 10 cases. J Cutan Pathol 1992; 19: 116-123.

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IDENTIFICATION OF A COMMON KERATIN 5 MUTATION IN EPIDERMOLYSIS BULLOSA SIMPLEX-WEBER-COCKAYNE.

Pamela Ehrlich, Virginia P. Sybert, Anne Spencer, Karen Stephens. University of Washington and Children's Hospital and Medical Center, Seattle, WA.

A mutation in codon 161 of the head domain of keratin 5 was recently identified in 2 unrelated patients with epidermolysis bullosa simplex-Weber-Cockayne (EBS-WC)(Chan et al, 1993). The mutation, a T to G transversion at basepair 873 (T873G) results in disruption of a *FokI* restriction site. To determine of this was a common mutation, we tested 13 unrelated probands with familial EBS-WC by PCR amplification and *FokI* digestion. Six probands tested positive and one was sequenced to confirm the T873G mutation. Analysis of their relatives confirmed cosegregation of T873G with EBS-WC phenotype. A 16 month old asymptomatic child of an affected parent was also tested and shown not to carry the mutation. Identification of this common mutation may be useful to confirm the diagnosis of EBS-WC in an at-risk asymptomatic individual of a family that carries the T873G mutation.

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EPIDERMOLYSIS BULLOSA DYSTROPHICA AND BLADDER EXTROPHY: Management of an uncommon association.

May El Hachem, Giuseppe Pianosi, Paolo Caione, Nicola Capozza, Tullio Faraggiana*, Annamaria Guadagni, Roberto Bianchi; "Bambino Gesù" Children's Hospital; Dept. Histopathology, University of Rome "La Sapienza"* - Rome, Italy.

Extrophy of urinary bladder and Epidermolysis Bullosa are quite rare diseases as single entities and present many complex clinical association in the same newborn terribly increases management problems.

In November 1993 at the Dept. of Dermatology of the "Bambino Gesù" Children's Hospital in Rome, Italy, we observed a case of two months old infant who was affected by both Epidermolysis Bullosa and Bladder Extrophy.

We could not find such association in the literature within last 10 years.

The infant was successfully operated for the severe urinary malformation combining intensive, neonatal and dermatological care.

Many problems that rised during the treatment and the long term follow-up of the baby will be presented at the symposium.

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THE NATIONAL EPIDERMOLYSIS BULLOSA REGISTRY - BASIC DEMOGRAPHIC CHARACTERISTICS OF A PROSPECTIVE MULTICENTER PROJECT. JD Fine, LB Johnson, H Tien, C Suchindran, EA Bauer, DM Carter, V Sybert, A Lin, D Caldwell-Brown, R Stern, J McGuire, A Spencer, S Gibbons, M Brust, L Nall, and A Moshell, National Epidermolysis Bullosa Registry, Chapel Hill, NC, Stanford, CA, New York, NY, Seattle, WA, & Bethesda, MD.

During its first 7 years, extensive data on 1764 patients with inherited and acquired epidermolysis bullosa (EB) were collected on behalf of the National Epidermolysis Bullosa Registry (NEBR), a multicenter, prospective epidemiological project. The mean age of enrollees was 22.1 yr (1st & 3rd quartiles, 4.3 & 35.6 yr). 52.7% were female, similar to the sex distribution within the US population. Overall, white non-Hispanics, blacks, and Hispanics comprised 86.0, 7.6, & 5.1% of the enrollee pool, respectively, although distinctive differences in ethnic distribution were noted when stratified by NEBR clinical center, consistent with geographic differences in the US population. The distribution of major EB subtypes on presentation and after complete evaluation at the NEBR was: EB simplex (EBS) - 49.9 & 50.7%; junctional EB (JEB) - 6.0 & 8.1%; dystrophic type unknown (DEB-U) - 6.2 & 2.0%; dominant dystrophic EB (DDEB) - 5.9 & 12.4%; recessive dystrophic EB (RDEB) - 13.3 & 15.6%; EB acquisita (EBA) - 1.3 & 1.2%; EB other type (EB-O) - 0.3 & 0.3%; EB type unknown (UNK) - 17.4 & 9.9%. This represents an overall change in diagnosis by major EB type of 26.3%. Excluding DEB-U, EB-O, and UNK categories, the greatest and least misclassifications were for DDEB (56.1%) and EBS (3.2%), although surprisingly 25.6% and 13.7% of severer forms (JEB & RDEB, respectively) were also underclassified prior to NEBR evaluation. On the basis of the collective data, it would appear that the current NEBR population has basic demographic characteristics representative of the US population, suggesting adequate sampling technique, and that correct diagnosis and subclassification of EB patients may be greatly influenced by physician experience and/or availability of sophisticated diagnostic tests.

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CLASSIFICATION AND REGRESSION TREE (CART) STATISTICAL TECHNIQUE FOR DIAGNOSIS OF MAJOR TYPES OF INHERITED EPIDERMOLYSIS BULLOSA (EB) - A SPLIT SAMPLE ANALYSIS OF THE NATIONAL EB REGISTRY DATASET. JD Fine, LB Johnson, H Tien, C Suchindran, EA Bauer, DM Carter, V Sybert, A Lin, D Caldwell-Brown, R Stern, J McGuire, A Spencer, S Gibbons, M Brust, L Nall, and A Moshell, National Epidermolysis Bullosa Registry, Chapel Hill, NC, Stanford, CA, New York, NY, Seattle, WA, & Bethesda, MD.

A recently described iterative biostatistical technique, referred to as CART, has been shown to have utility as a means of accurately identifying and classifying subsets within some larger populations. We have used this technique to determine the sensitivity [Se], specificity [Sp], and positive (PPV) and negative (NPV) predictive values of 8 clinical variables (enamel hypoplasia; milia; scars; pseudosyndactyly; esophageal stricture/ stenosis; growth retardation; other extracutaneous disease; nail dystrophy; excessive granulation tissue) to correctly identify one of 7 major EB types (EB simplex, Weber-Cockayne [WC]; EB simplex, other [EBS-O]; Herlitz junctional EB [JEB-H]; JEB, other [JEB-O]; dominant dystrophic EB [DDEB]; Hallopeau-Siemens RDEB [RDEB-HS]; RDEB, other [RDEB-O]). Our analyses involved a split sample technique, comparing data from one NEBR clinical center (referent pool) with that from the remaining other three. Both sample groups had identical Se for detection of JEB-H (1.0) and RDEB-HS (0.96), but Sp (1.0 vs 0.87; 0.98 vs 0.86) values were reduced in the non-referent group, substantially reducing PPVs for each EB type. In general, Se and Sp values within the non-referent group were less than those of the referent group for all other major EB subtypes. For example, the PPV for WC within the referent group was 0.88 but was only 0.65 in the non-referent group. Similarly, JEB-O was identified with Se=1.00, Sp=0.99, PPV=0.93, and NPV=1.0 in the referent group, but within the non-referent group Se=0.0, Sp=1.0; PPV=0.0, and NPV=0.90. The findings from this split sample study demonstrate lack of homogeneity within the two study subpopulations, reflecting either differences in the phenotypic features observed in some EB subsets examined at different geographic sites or the possible presence of misclassification bias as a result of differences in the manner in which some clinical features were defined.

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THE NATIONAL EPIDERMOLYSIS BULLOSA (EB) REGISTRY - RISK OF PREMATURITY, FREQUENCY OF PREGNANCY COMPLICATIONS, AND EFFECT OF MATERNAL AND PATERNAL AGES ON THE DEVELOPMENT OF EACH OF THE MAJOR EB TYPES. JD Fine, LB Johnson, H Tien, C Suchindran, EA Bauer, DM Carter, V Sybert, A Lin, D Caldwell-Brown, R Stern, J McGuire, A Spencer, S Gibbons, M Brust, L Nall, and A Moshell, National EB Registry, Chapel Hill, NC, Stanford, CA, New York, NY, Seattle, WA, and Bethesda, MD.

It is known that the risk of some congenital disorders is influenced by at least maternal age, and others may be associated with increased risk of premature birth. Nothing is yet known about the possible role of such factors in the development of inherited EB. We have therefore sought to address this issue by examining the reported frequencies for complications of pregnancy, the occurrence of premature versus full-term birth, and the distribution of maternal and paternal ages at the time of birth of affected offspring across four major EB types (EB simplex, EBS; junctional EB, JEB; dominant dystrophic EB, DDEB; recessive dystrophic EB, RDEB) represented prospectively in the National EB Registry (NEBR) cohort. Complications were reported by parents in 14.1, 21.0, 14.7, and 21.0% of pregnancies associated with the birth of EBS, JEB, DDEB, and RDEB patients, respectively. Whereas premature birth occurred at essentially the same frequency in association with EBS (6.0%), DDEB (4.2%), and RDEB (5.5%), 9.8% of all JEB infants were reported to have been born prematurely. Maternal and paternal ages at the time of birth of their affected children were grouped into five categories (<15; 15-19; 20-29; 30-39; ≥40 years of age) and then examined for possible differences in the distribution of ages. In general, no differences were noted either in distribution curves or in mean maternal ages (EBS, 26.2; JEB, 25.4; DDEB, 25.6; RDEB, 25.6 yr) or paternal ages (EBS, 28.6; JEB, 28.3; DDEB, 27.6; RDEB, 27.6 yr) when stratified by major EB type resulting from such a pregnancy. On the basis of these data, it is impossible to exclude the possibility that recall bias may have influenced the relative frequencies of reported pregnancy complications. Furthermore, these data by themselves do not exclude the possible influence of parental race, prenatal care, or socioeconomic status on the apparent increased risk of prematurity in JEB.

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THE NATIONAL EPIDERMOLYSIS BULLOSA (EB) REGISTRY - DIFFERENCES IN FREQUENCIES OF SELECTED GASTROINTESTINAL MANIFESTATIONS AND CANCERS ACROSS MAJOR DISEASE TYPES. JD Fine, LB Johnson, H Tien, C Suchindran, EA Bauer, DM Carter, V Sybert, A Lin, D Caldwell-Brown, R Stern, J McGuire, A Spencer, S Gibbons, M Brust, L Nall, and A Moshell, National EB Registry, Chapel Hill, NC, Stanford, CA, New York, NY, Seattle, WA, and Bethesda, MD.

The gastrointestinal tract (GI) appears to be among the most common sites of extra-cutaneous involvement in inherited EB, although data is as yet lacking as to the actual frequency of involvement in each of the major EB types. We have examined the frequencies of occurrence of 5 major GI findings (dysphagia; esophageal webbing or stenosis; pyloric stenosis; anal strictures; chronic constipation) in each of 4 well-characterized EB types (EB simplex, EBS; junctional EB, JEB; dominant dystrophic EB, DDEB; recessive dystrophic EB, RDEB) represented prospectively in the National EB Registry (NEBR) cohort. Whereas only 3.4% of EBS patients complained of dysphagia, 17.6, 14.2, and 58.1% of JEB, DDEB, and RDEB patients, respectively, noted this symptom. Esophageal webs or stenoses occurred in 0.4, 5.7, 2.6, and 38.0% of EBS, JEB, DDEB, and RDEB patients. Anecdotal data has suggested that pyloric stenosis or atresia may be seen in up to 15% of all JEB infants. Within the NEBR cohort, only 3.2% of JEB cases had associated pyloric stenosis, compared to 0.0, 0.0, and 0.4% of EBS, DDEB, and RDEB patients. Chronic constipation, a common symptom in many normal individuals, is believed to be a frequent problem in severe EB. This symptom was noted by 9.0% of EBS patients and 17.5, 16.7, and 46.7% of JEB, DDEB, and RDEB patients. Anal strictures were a rare event, occurring least often in EBS (0.5%) and most frequently in RDEB (5.7%). Neoplasms of the GI tract were rarely observed. Whereas no gastric cancers occurred, esophageal cancer was noted in 0.4% of RDEB cases, and intestinal cancers in 0.5 and 0.4% of EBS and RDEB cases, respectively. Collectively, these findings are consistent with the clinical impression that the GI tract, especially the esophagus, is a common site of injury in inherited EB, especially RDEB, although the risk of GI cancer remains very low in even the most severe forms of this disease.

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THE NATIONAL EPIDERMOLYSIS BULLOSA (EB) REGISTRY - DIFFERENCES IN FREQUENCIES OF EXTRACUTANEOUS INVOLVEMENT ACROSS MAJOR DISEASE TYPES. JD Fine, LB Johnson, H Tien, C Suchindran, EA Bauer, DM Carter, V Sybert, A Lin, D Caldwell-Brown, R Stern, J McGuire, A Spencer, S Gibbons, M Brust, L Nall, and A Moshell, National EB Registry, Chapel Hill, NC, Stanford, CA, New York, NY, Seattle, WA, and Bethesda, MD.

To assess the extent of extracutaneous disease activity in inherited EB, we compared the medical histories of four well characterized groups of EB patients (EB simplex, EBS; junctional EB, JEB; dominant dystrophic EB, DDEB; recessive dystrophic EB, RDEB) for evidence of one or more of the following major medical problems: anemia, growth retardation (GR), oral cavity abnormalities, mental retardation, and/or disease involve-ment of the gastrointestinal tract (GI), eyes, tracheolaryngeal (TL) tree, genitourinary (GU) tract, respiratory (RS) tree, cardiovascular (CV) system, and musculoskeletal (MS) system. In general, multiorgan involvement was primarily noted in JEB and RDEB. In most situations, little or no increase in frequency of positive signs or symptoms was noted for DDEB when compared to EBS. For example, an increased frequency in anemia was observed only in JEB (35.0%) and RDEB (48.5%). GI disease activity was reported in 75.3, 51.6, 28.3, and 20.4% of RDEB, JEB, DDEB, and EBS patients. The frequency of ocular tract involvement was increased only in RDEB (53.6%) and JEB (33.3%). Associated eye findings included corneal erosions (19.2%, JEB; 32.4%, RDEB), corneal scarring (9.7%, JEB; 19.1%, RDEB), symblepharons (3.6%, RDEB), blepharitis (8.5%, RDEB), ectropions (4.8%, JEB), and lacrimal duct obstruction (4.0%, JEB; 5.7%, RDEB). TL disease activity was confined to JEB (31.2%). Overall, 57.9% of RDEB patients experienced musculoskeletal involvement, most notably contractures (41.9%) and mitten deformities (45.7%). Significant intraoral involvement occurred in all major EB forms, ranging from 36.0% in EBS to 89.5% in RDEB. In contrast, no significant differences were noted in the frequency of GU, RS, or CV system abnormalities, or in mental retardation. These findings confirm that patients with JEB and RDEB are at particular risk for widespread extracutaneous disease activity, whereas for most patients with EBS and DDEB, morbidity is skin related.

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SKIN CANCER AND INHERITED EPIDERMOLYSIS BULLOSA (EB) - ANALYSIS OF THE NATIONAL EB REGISTRY COHORT BY DISEASE TYPE AND SUBTYPE. JD Fine, LB Johnson, H Tien, C Suchindran, EA Bauer, DM Carter, V Sybert, A Lin, D Caldwell-Brown, R Stern, J McGuire, A Spencer, S Gibbons, M Brust, L Nall, and A Moshell, National Epidermolysis Bullosa Registry, Chapel Hill, NC, Stanford, CA, New York, NY, Seattle, WA, and Bethesda, MD.

Case reports attest to the association of skin-derived squamous cell carcinoma (SCC) and recessive dystrophic EB (RDEB), but too few patients have been prospectively studied to permit estimation of the frequency of occurrence of such cancers. We have sought to ascertain the frequency of SCC, basal cell carcinoma (BCC), and malignant melanoma (MM) in 1568 enrollees in the National EB Registry. Whereas SCC was unobserved in junctional EB (JEB) and in only 0.12% (1/802) of EB simplex (EBS) patients, 1.02% and 9.02% of dominant dystrophic (DDEB) and RDEB patients, respectively, reported having had ≥ 1 SCC of skin origin. The odds ratio (OR) for the presence of ≥ 1 SCC in RDEB patients, compared to all other major types of EB, was 29.6; when EBS patients were used as a control population, the OR was 72.3. The highest frequency (14.5%) of SCCs was noted in the Hallopeau-Siemens RDEB variant; 9.5% of all other RDEB patients similarly developed SCC. In contrast, the risk of BCC was low, seen in only 1.26%, 0.0%, 2.07%, and 0.42% of EBS, JEB, DDEB, and RDEB patients, respectively. Presumably the reduced frequencies of BCC observed in JEB and RDEB reflect the high risk of premature death in both EB groups, thereby reducing the risk of a tumor associated with advanced age and longterm sunlight injury. Interestingly, in EBS, BCCs arose more commonly in the Dowling-Meara (4.6%) than Koebner (1.3%) or Weber-Cockayne (0.92%) variants. Within this limited cohort, MM was noted in only 3 patients, each of whom had EBS. Our findings confirm previous reports of an increased risk of skin-derived SCCs in RDEB, especially in patients with the severe generalized Hallopeau-Siemens variant. The striking differences in risk by histological type of skin tumor, as well as the usually young age of affected RDEB patients, further suggest that these SCCs arise by a mechanism other than that of chronic ultraviolet injury.

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THE NATIONAL EPIDERMOLYSIS BULLOSA REGISTRY - ASSESSMENT OF INTEROBSERVER VARIABILITY BY SENSITIVITY AND SPECIFICITY ANALYSIS OF TWO MAJOR CLINICAL PARAMETERS. JD Fine, LB Johnson, H Tien, C Suchindran, EA Bauer, DM Carter, V Sybert, A Lin, D Caldwell-Brown, R Stern, J McGuire, A Spencer, S Gibbons, M Brust, L Nall, and A Moshell, National Epidermolysis Bullosa Registry, Chapel Hill, NC, Stanford, CA, New York, NY, Seattle, WA, and Bethesda, MD.

The protean nature of epidermolysis bullosa (EB) suggests the likelihood of misdiagnosis when only clinical findings are considered. Subclassification is further hampered by the variability of findings within individual EB subtypes. Extensive data has now been collected on > 1700 EB patients by a limited number of physicians participating in the National EB Registry (NEBR) project. In the present study, we sought to measure interobserver variability by examining the sensitivity and specificity of exuberant granulation tissue (EGT) and pseudosyndactyly (SYN), since (a) EGT is believed to be pathognomonic for the Herlitz variant (H-JEB) of junctional EB, (b) SYN is considered to be characteristic of Hallopeau-Siemens (HS) recessive dystrophic EB, and (c) each should be readily identifiable by the practitioner. We determined findings for each within the entire dataset and stratified by NEBR clinical center. For analysis, three of the latter were then combined. We excluded from analysis any cases which could not be classified into at least one of the major EB types. Within the overall NEBR data, the presence of SYN was both sensitive (Se=93.8%) and specific (Sp=92.2%) for HS RDEB. Only minor differences in Se and Sp were noted when one center (96.4%; 96.4%) was compared to the other three (91.9%; 87.8%), suggesting agreement on the definition of SYN and similarities in the EB patient pools at the different clinical centers. When EGT was considered, analysis of the overall data suggested a Se and Sp of 64.3% and 95.5%, respectively, when used as a marker of H-JEB. When stratified by clinical centers, the reference center had Se and Sp of 100% each, whereas the other three centers had Se of only 37.5% and Sp of 90.5%. These latter data suggest that there may be considerable interobserver variability with regard to the definition of this particular clinical finding, suggesting the possibility of misclassification of some H-JEB patients.

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THE DIVERSITY OF CUTANEOUS AND EXTRACUTANEOUS FINDINGS IN EPIDERMOLYSIS BULLOSA (EB) SIMPLEX IS A FUNCTION OF SEVERITY OF DISEASE SUBTYPE. JD Fine, LB Johnson, H Tien, C Suchindran, EA Bauer, DM Carter, V Sybert, A Lin, D Caldwell-Brown, R Stern, J McGuire, A Spencer, S Gibbons, M Brust, L Nall, and A Moshell, National EB Registry, Chapel Hill, NC, Stanford, CA, New York, NY, Seattle, WA, & Bethesda, MD.

It is generally assumed that patients with EB simplex lack scarring, milia, nail dystrophy, intraoral blisters, and significant extracutaneous disease activity, although individual exceptions have repeatedly been observed. In order to determine the frequency of occur-rence of several of these findings we employed the extensive database of the National EB Registry, which contains data on the physical findings of 377 EB simplex (EBS) cases. We considered data on cases classified as Koebner (K), Dowling-Meara (DM), and Weber-Cockayne (WC) variants, and assessed 7 skin-associated parameters (scarring, milia, nail dystrophy, alopecia, hypotrichosis, pseudosyndactyly (SYN), contractures), presence or absence of intraoral blistering, and the extent of extracutaneous involvement (by the number (1; 2-5; ≥ 6) of positive responses for selected questions within the medical histories). Scarring was observed in 55.8, 34.4, and 10.5% of K, DM, and WC patients. Milia and nail dystrophy were seen in 15.4 & 57.7% of K, 28.1 & 68.8% of DM, and 4.2 & 11.4% of WC EBS patients, respectively. Most commonly observed in DM (6.2 & 9.4%), alopecia and hypotrichosis were also present in some patients with K (3.8 & 5.8%) and WC (0.8 & 0.4%) variants. Although absent in WC EBS, SYN and other contractures were noted in 9.4 & 15.6% of DM patients, and in 3.8 & 3.8% of K EBS. Intraoral blistering was seen in 62.5, 23.1, and 8.9% of DM, K, and WC patients. At least 1 positive response for significant extracutaneous disease was noted in 38.6, 18.4, and 4.6% of DM, K, and WC patients, and ≥ 2 responses in 15.9, 3.9, and 0.6%, respectively. These findings emphasize the fact that many of the cutaneous and intraoral findings believed to be present exclusively in dystrophic and, to a lesser extent, junctional EB, occur in each of the three major sub-types of EBS, and that in general, their frequency of occurrence (and that of extracutaneous disease activity) mirrors the extent and severity of cutaneous disease activity.

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THE PROTEAN NATURE OF CUTANEOUS MANIFESTATIONS IN INHERITED EPIDERMOLYSIS BULLOSA - ANALYSIS OF THE FREQUENCY OF FINDINGS BY MAJOR SUBTYPE OF DISEASE IN A LARGE, WELL-DEFINED COHORT OF PATIENTS. JD Fine, LB Johnson, H Tien, C Suchindran, EA Bauer, DM Carter, V Sybert, A Lin, D Caldwell-Brown, R Stern, J McGuire, A Spencer, S Gibbons, M Brust, L Nall, and A Moshell, National Epidermolysis Bullosa Registry, Chapel Hill, NC, Stanford, CA, New York, NY, Seattle, WA, and Bethesda, MD.

Common perceptions of the cutaneous features in inherited epidermolysis bullosa (EB) phenotypes have to date been based on published experiences with only limited numbers of patients. Using the extensive database of the National Epidermolysis Bullosa Registry, we sought to determine the frequency of several skin findings (scarring; milia; hypotrichosis; alopecia; nail dystrophy; syndactyly) for 5 major EB types - EB simplex (EBS), junctional EB (JEB), dystrophic EB subtype unknown (DEB-U), dominant dystrophic EB (DDEB), and recessive dystrophic EB (RDEB). Scarring, believed a constant feature in RDEB and DDEB, was seen in 95.7 & 91.5%, respectively, but was less often noted in DEB-U (66.7%), JEB (69.2%) and EBS (24.1%). Milia, also considered a characteristic marker of dystrophic EB, occurred in only 76.5, 60.0, and 50.0% of RDEB, DDEB, and DEB-U, respectively, and was also seen in some JEB (19.5%) and EBS (10.9%) cases. Nail dystrophy was also insensitive and nonspecific, occurring in 92.7, 82.6, 44.4, 89.7, and 27.7% of RDEB, DDEB, DEB-U, JEB, and EBS patients. Alopecia was primarily confined to RDEB (28.2%) and JEB (27.6%); hypotrichosis was almost exclusively seen in a minority of RDEB cases (10.5%). Pseudosyndactyly, considered a marker of RDEB, was observed in 61.8% of all RDEB patients, but also occurred in some cases with DDEB (0.8%), DEB-U (11.1%), JEB (6.5%), and EBS (1.3%). Some cutaneous findings, notably scarring and milia, may not be present at or shortly after birth in even severer forms of EB, resulting in an underestimate of their true frequency of occurrence over time. However, these findings emphasize the considerable overlap in cutaneous findings observable across the spectrum of major EB types, and emphasize the need for the performance of sophisticated diagnostic tests, in addition to physical examination and medical and genetic histories, for proper diagnosis.

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THE NATIONAL EPIDERMOLYSIS BULLOSA REGISTRY - DIFFERENCES BY MAJOR AND MINOR EB TYPE IN AGE OF ONSET, AGE AND MEANS OF DIAGNOSIS, AND PRESENCE OR ABSENCE OF SIGNIFICANT ASSOCIATED EXTRACUTANEOUS DISEASE . JD Fine, LB Johnson, H Tien, C Suchindran, EA Bauer, DM Carter, V Sybert, A Lin, D Caldwell-Brown, R Stern, J McGuire, A Spencer, S Gibbons, M Brust, L Nall, and A Moshell, National Epidermolysis Bullosa Registry, Chapel Hill, NC, Stanford, CA, New York, NY, Seattle, WA, and Bethesda, MD.

Each major type of inherited epidermolysis bullosa (EB), as reflected in the data collected prospectively on 1764 enrollees in the National Epidermolysis Bullosa Registry (NEBR), had its mean age for onset of clinical activity within the first 7 months of life (earliest, 0.05 yr, junctional EB; latest, 0.61 yr, EB simplex), whereas the mean age at diagnosis ranged from 1.57 yr (recessive dystrophic EB) to 7.72 yr (EB simplex). The most commonly employed diagnostic test was electron microscopy (EM), ranging in use from 10.6% of EBS cases to 44.0% in RDEB. Major EB subtypes differed considerably in the presence and extent of extracutaneous involvement (per history), with 8.7, 62.8, 18.2, and 73.4% of EB simplex (EBS), junctional EB (JEB), dominant dystrophic (DDEB), and recessive dystrophic EB (RDEB), respectively, reporting extracutaneous (excluding intraoral) disease activity. Further differences were noted when stratification was performed by EB subtype. Whereas only 4.6% of Weber-Cockayne EBS had associated extracutaneous disease, the latter was present in 18.4 and 38.6% of Dowling-Meara and Koebner patients, respectively. Similar-ly, 75.0 and 87.5% of gravis (Herlitz) and mitis JEB patients gave a positive history, as did 25.0, 20.6, 97.7, 88.8, and 53.6% of patients with Cockayne-Touraine DDEB, Pasini DDEB, Hallopeau-Siemens RDEB, inversa RDEB, and other forms of RDEB. In general, these findings confirm clinical impressions which are found within the medical literature for each of the major EB types, in that those EB subsets known to usually have more severe cutaneous disease activity do have earlier mean age of onset and a higher frequency of extracutaneous involvement. More careful analysis, however demonstrates a wider range of variation for these parameters than previously appreciated. In particular, extracutaneous disease may occur in a significant minority of even the more generalized forms of EB simplex.

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STATISTICAL CLASSIFICATION MODELS FOR INHERITED EPIDERMOLYSIS BULLOSA (EB) - COMPARISON OF THE INFLUENCE OF DIFFERENT CLINICAL VARIABLES ON SENSITIVITY AND SPECIFICITY. JD Fine, CM Suchindran, H Tien, LB Johnson, and A Moshell, Departments of Biostatistics, Dermatology, and Epidemiology, Schools of Public Health and Medicine, University of North Carolina at Chapel Hill, the Southeastern Clinical Center of the National EB Registry (NEBR), Chapel Hill, NC, and the National Institutes of Health, Bethesda, MD.

Data is lacking on the relative utility of clinical findings in both diagnosis and subclassification of patients with inherited EB, although it would appear that significant overlap in some phenotypic features does occur across major and minor EB types. In order to try to develop clinically useful algorithms for diagnosis, we have employed a complex iterative statistical technique, CART (Classification and Regression Trees), and have attempted to correctly predict one of 7 major EB types (EB simplex, Weber-Cockayne [WC]; EB simplex, other [EBS-O]; Herlitz junctional EB [JEB-H]; JEB, other [JEB-O]; dominant dystrophic EB [DDEB]; Hallopeau-Siemens RDEB [RDEB-HS]; RDEB, other [RDEB-O]) using 7 shared clinical variables (enamel hypoplasia; milia; scars; pseudosyndactyly; esophageal stricture/stenosis; growth retardation; other extracutaneous disease). In model 1, tracheolaryngeal disease served as an 8th variable, whereas in model 2, nail dystrophy and excessive granulation tissue were added. To prevent interobserver variability, data were confined to that of the NEBR Southeastern Clinical Center. Both models were identical in their ability to accurately identify JEB-H (sensitivity [Se] & specificity [Sp] for each model = 1.0), JEB-O (Se=1.0; Sp=0.98-0.99), and RDEB-HS (Se, 0.96; Sp, 0.98). Whereas both models had equal Se (0.96) for diagnosing WC, Sp decreased with model 2 (0.90 vs 0.80). Although model 2 increased the Sp of identifying EBS-O (1.0 vs 0.85), Se dropped from 0.56 to 0.0. Both models had high Sp for RDEB-O (0.99 & 1.0) but were very insensitive (model 1, 0.24; model 2, 0.0). However, model 2 did increase Se (0.98 vs 0.56) of identifying DDEB at minor expense of Sp (0.78 vs 0.89). These data demonstrate that collections of selected clinical parameters can be used to accurately identify major EB subtypes, but that the addition of at least one variable, nail dystrophy, may alter Se and Sp.

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RISK OF ESOPHAGEAL WEBBING OR STENOSIS IN INHERITED EPIDERMOLYSIS BULLOSA (EB): ASSESSMENT BY LIFETABLE ANALYSIS TECHNIQUE. JD Fine, LB Johnson, H Tien, C Suchindran, L Brock, EA Bauer, DM Carter, V Sybert, A Lin, D Caldwell-Brown, R Stern, J McGuire, A Spencer, S Gibbons, M Brust, L Nall, and A Moshell, National Epidermolysis Bullosa Registry, Chapel Hill, NC, Stanford, CA, New York, NY, Seattle, WA, & Bethesda, MD.

It is known that esophageal webbing or stenosis (EWS) occurs in recessive dystrophic EB (RDEB), and that each may be associated with significant morbidity. Although as yet unproven, the presence of persistent partial obstruction of the esophagus may contribute to the altered nutritional status of some EB patients, influencing growth, development, and/or wound healing. Data is yet lacking about the time of onset of EWS in RDEB, as well as the relative likelihood of this finding in other forms of EB. In order to address both, we have performed lifetable analysis, utlizing data on 1407 consecutive EB cases (EB simplex [EBS], 862; junctional EB [JEB], 130; dominant dystrophic EB [DDEB], 202; RDEB, Hallopeau-Siemens [RDEB-HS], 77; RDEB, other [RDEB-0], 136) within the National EB Registry. By age 1, the probability of EWS was 5.30% and 3.94% for RDEB-HS and RDEB-O patients, respectively. In contrast, the probability of EWS was only 0.12%, 0.88%, and 0.00% in EBS, JEB, and DDEB. By age 3, the probability of EWS was 10.88% and 7.52% in RDEB-HS and RDEB-O patients, respectively, and 2.08% in JEB. By ages 5 and 10, the probability of EWS was 23.79% and 37.74% in RDEB-HS, and 8.67% and 12.96% in RDEB-O. In comparison, by age 10, the probability of EWS was 0.12%, 5.36%, and 0.74% in EBS, JEB, and DDEB. By ages 25 and 30, the probabilities of EWS in RDEB-HS were 66.03% and 79.62%, and in RDEB-O were 30.66% and 34.13%, respectively. Similarly, the probability of EWS in JEB by age 30 was 9.86%, but only 0.30% and 0.74% in EBS and DDEB. These findings emphasize that the EWS may occur in a significant minority of RDEB patients, especially those with the H-S variant, as early as 1 yr of age, and that about half and three-quarters of all RDEB-HS patients will likely experience EWS by ages 15 and 30, respectively. EWS also occurs in RDEB-O, although a lower probability is observed at each age interval when compared to the H-S subtype. Interestingly, about 10% of JEB patients are also at risk of EWS by age 30 whereas the probability of EWS in DDEB is only slightly greater than that of EBS. Based on these collective findings, patients with RDEB, especially those with the H-S variant, should be closely monitored during early childhood for possible EWS.

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PSEUDOSYNDACTYLY (MITTEN DEFORMITIES) AND INHERITED EPIDERMOLYSIS BULLOSA (EB): TIME TO ONSET IN MAJOR EB SUBSETS AS DETERMINED BY LIFETABLE ANALYSIS TECHNIQUE. JD Fine, LB Johnson, H Tien, C Suchindran, L Brock, EA Bauer, DM Carter, V Sybert, A Lin, D Caldwell-Brown, R Stern, J McGuire, A Spencer, S Gibbons, M Brust, L Nall, and A Moshell, National Epidermolysis Bullosa Registry, Chapel Hill, NC, Stanford, CA, New York, NY, Seattle, WA, & Bethesda, MD.

Pseudosyndactyly (SYN) is a known complication of the Hallopeau-Siemens (HS) subtype of recessive dystrophic EB (RDEB) and has been reported in one rare junctional EB (JEB) variant (cicatricial JEB). Most clinicians include SYN as a major criterion in the diagnosis of RDEB-HS. Data is yet lacking about the frequency of occurrence and the time of onset of SYN in RDEB-HS, as well as the relative likelihood of this finding in other forms of EB. In order to address both, we have performed lifetable analysis, utilizing data on 1395 consecutive EB cases (EB simplex [EBS], 863; junctional EB [JEB], 131; dominant dystrophic EB [DDEB], 211; RDEB, Hallopeau-Siemens, 56; RDEB, other [RDEB-O], 134) within the National EB Registry on whom data existed as to both presence or absence of SYN and its date of onset. By 1 yr of age, the probability of SYN was 16.67% and 7.26% in patients with RDEB-HS and RDEB-O, respectively. By ages 2, 3, 4, and 5, the probabilities of SYN in RDEB-HS were 28.43%, 44.34%, 54.46%, and 71.02%, respectively. In contrast, the probability of SYN was 12.22% in RDEB-O, as well as 1.77%, 0.12%, and 0.00% in JEB, EBS, and DDEB patients, by age 5. By ages 10, 20, and 30, the probabilities of SYN were 85.29%, 88.97%, and 93.38% in RDEB-HS. In comparison, the probabilities were 24.60%, 5.35%, 1.11%, and 0.12% in RDEB-O, JEB, DDEB, and EBS patients, respectively, age 30. On the basis of these findings, it is clear that SYN is an early feature in RDEB-HS, with probabilities of over 25%, 50%, and 75% by ages 2, 4, and 7, respectively. However, the probability of SYN in RDEB-HS by age 30 was only 93%, demonstrating that the absence of SYN in no way excludes the diagnosis of RDEB-HS. Interestingly, by age 30 about 25% of all patients classified as having RDEB-O were at risk of having developed SYN, also suggesting some lack of specificity of SYN as a diagnostic marker of HS. SYN is a rare feature in other forms of inherited EB, although the probability of SYN is about 5% in all JEB patients by as early as age 10, confirming its previous description in a rare JEB subtype. Knowing the early age of onset of SYN in RDEB-HS and realizing its serious implication on functionality, clinicians should anticipate its occurrence and consider implementing any measures which may either postpone the onset or limit the extent of SYN.

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TRACHEOLARYNGEAL STENOSIS AND INHERITED EB (EB): DETERMINATION OF DIFFERENCES IN TIME OF ONSET, AS ASSESSED BY LIFETABLE ANALYSIS TECHNIQUE. JD Fine, LB Johnson, H Tien, C Suchindran, L Brock, EA Bauer, DM Carter, V Sybert, A Lin, D Caldwell-Brown, R Stern, J McGuire, A Spencer, S Gibbons, M Brust, L Nall, and A Moshell, National Epidermolysis Bullosa Registry, Chapel Hill, NC, Stanford, CA, New York, NY, Seattle, WA, & Bethesda, MD.

Tracheolaryngeal stenosis or obstruction (TSO) can be a life-threatening occurrence in inherited EB. It is believed that TSO is confined to junctional EB (JEB) but it is unknown whether any differences exist among JEB subtypes as to either risk or outcome of TSO. Anecdotal experience also suggests that TSO is a complication of EB that occurs exclusively during infancy or early childhood. Some investigators have further suggested that the risk of TSO in JEB patients is confined to the first two years of life. Data is also lacking as to the relative likelihood of TSO in other forms of EB. In order to address these issues, we have performed lifetable analysis, utlizing data on 1454 consecutive EB cases (EB simplex [EBS], 863; Herlitz junctional EB [JEB-H], 18; non-Herlitz JEB [JEB-O], 108; dominant dystrophic EB [DDEB], 210; RDEB, 254) within the National EB Registry on whom data existed as to both presence or absence of TSO and its date of onset. With the exception of 1 case of TSO in 254 RDEB patients which occurred within the first year of life (estimated risk = 0.41%), TSO was confined to JEB. By age 1, the probability of TSO was 5.71% and 6.52% in JEB-H and JEB-O cases, respectively. By ages 2, 3, and 5, the probabilities of TSO were 11.61%, 18.41%, and 18.41% in JEB-H, in contrast to 9.33% (for ages < 5) for JEB-O. On or after age 6, the probability of TSO was 27.47% and 11.03% in JEB-H and JEB-O patients, respectively. There were no JEB-H patients surviving to or after age 20 on whom sufficient data existed to permit further follow-up on the occurrence of TSO, although no further episodes of TSO occurred in JEB-O patients on whom data existed through age 65. These collective data confirm the specificity of TSO for JEB and demonstrate that only a minority of JEB patients are at risk. Whereas the risk of TSO appears to be equal for both major JEB subtypes through age 2, almost twice as many JEB-H patients are at risk, compared to JEB-O, on or after age 3. Although no further occurrences of TSO were noted after age 6, the probability of TSO in both groups increased until that age. As such, it is important that clinicians realize that TSO may first present at a later time than that suggested in the current literature. Furthermore, although the total number of JEB-H patients followed in this study does limit the power of interpretation of our results, it does appear that patients with JEB-H are at higher risk than JEB-O, after early childhood, for TSO.

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RISK OF SKIN CANCERS AND INHERITED EPIDERMOLYSIS BULLOSA (EB) -- DETERMINATION OF DIFFERENCES ACROSS MAJOR EB SUBTYPES, AS ASSESSED BY LIFETABLE ANALYSIS TECHNIQUE. JD Fine, LB Johnson, H Tien, C Suchindran, L Brock, EA Bauer, DM Carter, V Sybert, A Lin, D Caldwell-Brown, R Stern, J McGuire, A Spencer, S Gibbons, M Brust, L Nall, and A Moshell, National Epidermolysis Bullosa Registry, Chapel Hill, NC, Stanford, CA, New York, NY, Seattle, WA, & Bethesda, MD.

It is known that some patients with severe generalized recessive dystrophic EB (RDEB) develop biologically aggressive squamous cell carcinomas (SCCs) of the skin; in some, fatality may result. Data is lacking on the cumulative risk over time of developing SCCs for each of the major EB subtypes, as well as the risk of developing other skin tumors. To address this, we have performed lifetable analysis, utlizing data on 1460 consecutive EB cases (EB simplex [EBS], 865; Herlitz junctional EB [JEB-H], 19; non-Herlitz JEB [JEB-O], 116; dominant dystrophic EB [DDEB], 208; RDEB, Hallopeau-Siemens variant [RDEB-HS], 100; RDEB, other [RDEB-O], 152) within the National EB Registry on whom data existed as to both presence or absence of at least one skin tumor and its date of onset. We considered 5 groups of skin tumors: all tumors combined, SCCs, basal cell carcinomas (BCCs), malignant melanomas (MMs), and other skin tumors. In general, significant probabilities for developing a skin tumor by age 30 were observed only in RDEB-HS and RDEB-O, occurring in 52.9% and 26.3%, respectively. SCCs were seen exclusively in these two RDEB groups, with a probability of occurrence of 1.3% by age 10 in RDEB-O. By age 20, probabilities of ≥ 1 SCC were 5.6% and 4.8% in RDEB-HS and RDEB-O. Whereas the probability of ≥ 1 SCC rose to 19.0% in RDEB-O by age 35, the probabilities in RDEB-HS were 23.7%, 43.4%, and 51.0% by ages 25, 30, and 35, respectively. MMs were seen only in RDEB-HS, occurring as early as age 4 (probability = 1.1%). By age 12, the probability of MM was 4.1%. In contrast, no EB patients were found to have BCCs before age 20; by age 35, the only EB subtype experiencing BCCs was DDEB (probability = 1.1%). Other types of skin tumors were essentially confined to RDEB-O, with probabilities of occurrence in 0.8%, 2.3%, 5.0%, and 9.0% of patients by ages 5, 15, 30, and 35, respectively. These data suggest that surveillance for SCC should begin during mid-childhood and late adolescence in patients with RDEB-O and RDEB-HS, respectively, and continue for the remainder of their lives. Finally, MM rarely does occur in RDEB-HS, and may develop very early in life, further suggesting the need for careful monitoring of all pigmented lesions.

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RISK OF PREMATURE DEATH AND INHERITED EPIDERMOLYSIS BULLOSA (EB) -- ASSESSMENT BY LIFETABLE ANALYSIS ACROSS MAJOR EB TYPES AND SUBTYPES OF PATIENTS WITHIN ONE REGIONAL CENTER OF THE NATIONAL EPIDERMOLYSIS BULLOSA REGISTRY (NEBR). JD Fine, LB Johnson, H Tien, C Suchindran, L Brock,and A Moshell, Departments of Dermatology, Biostatistics, and Epidemiology, Schools of Medicine and Public Health, University of North Carolina at Chapel Hill, NEBR Southeastern Clinical Center, Chapel Hill, NC, and the National Institutes of Health, Bethesda, MD.

It is known that potentially any form of inherited EB, especially those with generalized cutaneous disease activity, may result in death during infancy or early childhood. In addition, it is known that many patients with the Hallopeau-Siemens variant of recessive dystrophic EB (RDEB-HS) develop life-threatening squamous cell carcinomas (SCCs) beginning as early as the second decade of life. Precise data is lacking, however, as to the risk of premature death over time, either by major EB type or subtype. To address this, we have performed lifetable analysis, utlizing data on 923 EB cases (EB simplex, Dowling-Meara [EBS-DM], 16; EBS, other [EBS-O], 590; Herlitz junctional EB [JEB-H], 11; non-Herlitz JEB [JEB-O], 63; dominant dystrophic EB [DDEB], 132; RDEB-HS, 51; RDEB, other [RDEB-O], 60) within the NEBR Southeastern Clinical Center on whom up to 7 yrs of longitudinal data existed on the occurrence and date of death. All causes of death were included in the analysis. When only the major EB types (EBS, JEB, DDEB, RDEB) were considered, premature mortality was observed with only JEB and RDEB. By 1 yr of life, the probability of death was 37.41% in JEB. This probability increased to 45.65% by age 5 and remained unchanged through age 40. Whereas no mortality was noted in RDEB at ages ≤ 5 yr, the cumulative probability of death in RDEB was 1.39%, 9.96%, 21.71%, and 33.31% by ages 10, 25, 35, and 40, respectively. When further stratified by EB subtype, mortality in RDEB was confined to HS patients. A much higher probability of death was noted in non-Herlitz than Herlitz JEB patients at all comparable ages, although this likely reflects inclusion of indeterminant JEB subtypes within the former subpopulation, many of whom would have presumably been later classified as JEB-H on the basis of the appearance of more characteristic phenotypic features had longer survival been possible. These findings demonstrate that both major JEB subtypes are at great risk of death within the first few years of life, whereas increased risk of mortality in RDEB-HS is primarily a problem in the mid-twenties, consistent with the known timing of SCCs in the latter patients.

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FURTHER EVIDENCE THAT IMPAIRED EXPRESSION OF LAMC2 GENE IS INVOLVED IN HERLITZ JUNCTIONAL EPIDERMOLYSIS BULLOSA. M.F. Galliano[1], C. Baudoin[1], G. Tadini[2], R. Cavalli[2], A. Brusasco[2], G. Meneguzzi[1] and J.-P. Ortonne[1]. [1]U385 INSERM, Faculté de Médecine, Nice, France, [2]Center for Inherited Cutaneous Diseases, University of Milan, Italy.

Mutations in the gene encoding the γ2 chain (LAMC2) of laminin-5 (nicein/kalinin) have recently been demonstrated in forms of junctional epidermolysis bullosa (JEB). Two distinct cases of Herlitz JEB (H-JEB) were associated with a single base substitution creating a premature termination of translation and a severe reduction in the amount of mutant allele transcript. To better define the spectrum of mutations occurring in Herlitz JEB patients, we have undertaken a survey of a number of independent affected kindreds. We report here the characterization of a new H-JEB kindred in which the clinical phenotype of two affected members born from a consanguineous union has been associated with an impaired synthesis of laminin γ2 chain. Immunofluorescence studies of the skin of the affected patients, performed with polyclonal antibodies elicited against each subunit of laminin-5, revealed absence of immunoreactivity for laminin γ2 chain. A maximum two-point lod score of 1.40 at θ=0 was observed between a microsatellite near the LAMC2 gene and the disease. Northern blot analysis of RNA isolated from primary keratinocyte cultures from one of these patients did not detect hybridization with cDNAs encoding laminin γ2 chain. These results indicate that the lethal H-JEB phenotype is associated with a homozygous mutation probably resulting in premature termination codon. The search for the genetic alteration in this H-JEB kindred is in progress.

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KINDLER'S SYNDROME - CLINICAL ULTRASTRUCTURAL FINDINGS.

RM Haber and WM Hanna, Division of Dermatology and Department of Pathology, Women's College Hospital, University of Toronto, Canada.

We present two brothers with Kindler's syndrome. Both had a history of primarily acral blistering since infancy as well as photosensitivity. The brothers showed poikilodermatous changes on the elbows, knees and dorsum of the hands with mild cutaneous atrophy.

The younger brother (age 21) had actinic keratoses on the dorsum of his wrist which has not been previously described in Kindler's syndrome.

Ultrastructural examination was done on 3 biopsies. All showed bulla formation through the basal keratinocytes. This was associated with degenerative change of keratinocytes and marked increase and clumping of tonofilaments in the intact cells. The hemidesmosomes and anchoring fibrils had a normal morphology. There was duplication of the lamina densa, dermal fibrosis and marked actinic degeneration of the elastic fibers. These findings were demonstrated in both the spontaneous and induced blister in the younger brother and in an induced blister in the older brother. These ultrastructural findings have not been previously reported in patients with Kindler's syndrome.

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COLLAGEN DRESSING THERAPY FOR A PATIENT WITH EPIDERMOLYSIS BULLOSA--A CASE PRESENTATION. S Harkins, Enterostomal Therapy Service, Waterbury Hospital Health Center, Waterbury, CT 06708.

An ET nurse uses a poster presentation to make a follow-up reporting of an effective response achieved as a newly formulated dressing was used to treat a patient with Recessive Dystrophic Epidermolysis Bullosa after conventional therapies failed. Skin Temp is a unique composite nylon mesh to which a porous collagen membrane is attached. The collagen can remain in contact with the wound and the nylon peeled off without disturbing the wound. Application of pressure to the nylon edges increases pore size in the nylon allowing exudate to flow to the surface where it can be easily wiped off. This material has limited permeability and is biocompatible. Medifil consists of spherical hydrophilic particles of collagen, 0.1 to 0.3 mm in diameter. These particles are available as a paste, powder and non-adherent pad. The subject of this work is a 30 year-old woman who had been home-bound because of huge wounds about her trunk. Non-healing persisted despite serial course of topical medicines, clear dressing, hydrocolloid dressings, and cadaveric skin grafts. With the concurrence of the patient's plastic surgeon and with the patient's informed consent, this ET nurse treated the patient with collagen dressings. The collagen dressings were comfortably tolerated by the patient and were readily handled by visiting nurses. The patient's wounds healed completely within six months of collagen dressing treatments. This ET nurse did serial evaluations prior to, during, and after the collagen dressing treatments. Photographic documentation depict results of the use of the collagen 8 months later. Collagen dressing treatments elicited comfortable, prompt, and persisting healing of the huge wounds in this patient.

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CHARACTERIZATION OF LAMININ 5 (NICEIN/ KALININ) B CHAIN DEFECTS IN JUNCTIONAL EPIDERMOLYSIS BULLOSA AND DEVELOPMENT OF GENE THERAPY APPLICATIONS. Warren Hoeffler, Scott Herron, Chihiro Matsui, Caroline Lanigan, Kevin Hultquist, Charlotte Cantrell, German Hernandez, Gordon Lee, and Eugene Bauer. Department of Dermatology, Stanford University School of Medicine, Stanford, CA.

The genes for laminin 5 subunits are candidates for the skin fragility observed in Recessive Junctional Epidermolysis Bullosa (RJEB). Our laboratory has screened twenty patients with RJEB of varying clinical phenotypes for DNA polymorphisms in laminin 5 B chain genes. RT-PCR amplification of B chain mRNAs served as templates for Single Stranded Conformational Polymorphism (SSCP) and Mutation Detection Enhancement (MDE) gel electrophoresis. Eleven putative polymorphisms were detected in B chain genes from different patients. These were characterized further by subcloning and sequencing of DNA products. Two candidate polymorphisms of functional significance were chosen for development of possible gene therapy applications. Delivery of both normal and mutant genes through *ex vivo* and *in vivo* techniques are being pursued. Parameters considered crucial to the introduction of a normal therapeutic gene include the following: development of appropriate eukaryotic expression vectors, optimization of transfection conditions, culturing of patients cells for delivery. We evaluated three promoter systems to drive the expression of B chain genes. Firstly, we established a constitutive expression system capable of producing high levels of B chains ($\beta 3$ and $\gamma 2$). We then evaluated an inducible expression system dependent upon tetracycline levels, and lastly we assessed tissue specific activity of the keratin 5 promoter for basal keratinocyte expression. The coupling of methods used for the identification of genetic lesions and the introduction of expression vectors into patient cells should provide the foundation for correcting the RJEB phenotype.

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LARYNGEAL INVOLVEMENT IN DOWLING-MEARA EPIDERMOLYSIS BULLOSA SIMPLEX. H.M. Horn, A.I.G. Kerr and M.J. Tidman, Departments of Dermatology and Otolaryngology, University of Edinburgh, Scotland, United Kingdom.

A full term male infant born to non-consanguinous parents with no family history of bullous disorders, developed widespread blistering of the skin and buccal mucous membranes within twenty-four hours of birth. Ultrastructural examination of a blister showed cytolysis of basal keratinocytes and tonofilament clumping characteristic of the Dowling-Meara variant of epidermolysis bullosa simplex. The child's voice remained persistently hoarse. Direct laryngeal examination at six months of age showed raised irregular lesions on both vocal cords. Ultrastructural examination of a biopsy from the right vocal cord showed features identical to those present in the skin, with a cleavage plane through the subnuclear portion of the basal epithelial cells and clumping of tonofilaments.

We have subsequently seen a second infant with Dowling Meara EB simplex with pronounced hoarseness of the voice but a laryngeal biopsy was not undertaken.

Hoarseness is usually considered characteristic of the junctional form of epidermolysis bullosa. These two cases demonstrate that it may also be a feature of Dowling-Meara EB simplex.

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A MUTATION IN THE SPLICE DONOR SITE OF INTRON 1 IN THE KERATIN 5 GENE IN THE DOWLING-MEARA FORM OF EPIDERMOLYSIS BULLOSA SIMPLEX. A. Hovnanian[1], M-O. Préhu[1], I. Guillemin[1], C. Blanchet-Bardon[2], A. Rochat[3], F. Gosselin[4], Y. Barrandon[3] and M. Goossens[1]. [1]Department of Genetics, Hôpital H. Mondor, Créteil, [2] Department of Dermatology, Hôpital St-Louis, Paris. [3]Ecole Normale Supérieure, Paris. [4]Collège de France, Paris, France.

Epidermolysis bullosa simplex (EBS) has been shown to arise from mutations within the keratin 5 and 14 genes. Most of these are missense mutations, although a three nucleotide deletion in the keratin 14 gene was recently reported by M.A. Chen *et al.* (Hum. Mol. Genet. 1993, 2:1971-1972). We investigated a family with 11 individuals dominantly affected with the Dowling-Meara form of EBS and searched for defects within the keratin 5 and 14 genes. Screening for mutations within reverse-transcribed PCR products from cultured keratinocytes from an affected member, revealed a 66 basepair deletion within the keratin 5 cDNA. This defect predicts an inframe deletion of the last 22 amino acid residues of exon 1 of keratin 5, which encompasses the last 8 amino acids of the head domain, and the first 14 amino acids of the rod domain, thus including the helix initiation peptide. Sequencing of PCR-amplified genomic DNA showed a heterozygous G-to-A substitution within the splice donor site of the first intron of the keratin 5 gene, but no deletion. The examination of exon 1 revealed a GTGAG consensus sequence at the 5' end of the deleted fragment which may have become functional as an alternative splice donor. This mutation is the only defect identified in the keratin 5 cDNA sequence, and cosegregates with the disease. Due to the functional importance of the deleted region, our data strongly suggests that this mutation is the underlying cause of the disease in this family.

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RECURRENT NONSENSE MUTATIONS WITHIN THE TYPE VII COLLAGEN GENE IN PATIENTS WITH SEVERE RECESSIVE DYSTROPHIC EPIDERMOLYSIS BULLOSA. A Hovnanian[1], L Hilal[1], C Blanchet-Bardon[2], Y de Prost[3], AM Christiano[4], J Uitto[4], and M Goossens[1], [1]Department of Genetics, Hôpital H Mondor, Créteil, [2]Department of Dermatology, Hôpital St-Louis, Paris, [3]Department of Dermatology, Hôpital Necker-Enfants Malades, Paris, France, [4]Departments of Dermatology and Molecular Biology, Jefferson University, Philadelphia, Pennsylvania, USA.

We investigated 52 unrelated patients affected with the Hallopeau-Siemens form of recessive dystrophic epidermolysis bullosa (HS-RDEB) and 2 patients with RDEB inversa for the presence of mutations within the type VII collagen gene (COL7A1). We selectively screened for mutations at CpG dinucleotides changing CGA arginine codons to premature stop codons TGA. Mutation analysis was performed using denaturing gradient gel electrophoresis (DGGE), followed by direct sequencing of PCR-amplified products. This strategy led to the characterization of a C-to-T transition at arginine codon 109 in one COL7A1 allele in two patients: one affected with HS-RDEB and the other with RDEB inversa. Two other HS-RDEB patients have a heterozygous C-to-T transition at arginine 1213 and 1216, respectively. These nonsense mutations predict the truncation of the collagenous and the non-collagenous NC-2 domains of the polypeptide, and thus are likely to lead to non-functional molecules. On the basis of linkage analysis which showed no evidence for locus heterogeneity in HS-RDEB, the second defect is likely to lie within the other COL7A1 allele. These results indicate that stop mutations within the COL7A1 gene can underlie both HS-RDEB and RDEB inversa, thus providing further evidence for the implication of this gene in RDEB.

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ABSENCE OF KERATIN 14 AND OF 10-NM INTERMEDIATE FILAMENTS IN BASAL CELLS IN AUTOSOMAL RECESSIVE EPIDERMOLYSIS BULLOSA SIMPLEX HERPETIFORMIS.

Marcel F. Jonkman*, Marcelus C.J.M. de Jong*, Klaas Heeres*, Marja J.A. van Luyn†, Jan B. van der Meer*, Departments of *Dermatology and †Medical Electron Microscopy, University Hospital, Groningen, the Netherlands.

We studied a family with epidermolysis bullosa simplex herpetiformis with autosomal recessive trait of inheritance. Immunofluorescence microscopy of clinically normal skin of the affected members showed no binding with moabs RCK107 and LL002 to keratin 14 in the basal cell layer, whereas staining to keratin 5 with moabs RCK102 and AE14 was normal. The suprabasal keratines 1+10 (CK8.60) were normal expressed, as well as the 230-kD (815) and 180-kD (1D1) bullous pemphigoid antigens, and the 500-kD plaque protein HD-1 (HD-121).

Electron microscopy of lesional skin revealed an intra-epidermal split with cytolysis of the basal cells. Basal cells in specimens from clinically normal skin were electron-lucent and completely lacked 10-nm intermediate filaments. The suprabasal cells had normal intermediate filaments with some filamentous aggregations in the cytoplasm. Hemidesmosomes and other basement membrane structures were normal. Remarkably the basal cells retained, in the absence of the tonofilament cytoskeleton, a normal undulating basal outline with bulging micro-projections (rootlets) into the dermis. At high power magnification we found loose 6-nm filaments parallel with the cell membrane, that randomly inserted into the cell membrane, partly also into hemidesmosomes. Immuno-electron microscopy will be performed to reveal their identity: actin microfilaments or perhaps keratin protofibrils?

This is the first study describing the combination of three rare features in a family with epidermolysis bullosa simplex: autosomal recessive trait of inheritance, no keratin 14 expression and lack of intermediate filaments in the basal keratinocytes. Keratin 14 obligatory pairs with keratin 5 into heterodimers (coiled-coil). In this subtype of epidermolysis bullosa simplex, assembly of intermediate filaments in the basal keratinocytes has apparently ceased, due the absence or severe deficiency of keratin 14 polypeptides.

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180-KD BULLOUS PEMPHIGOID ANTIGEN (BP180) IS DEFICIENT IN A PATIENT WITH GENERALIZED ATROPHIC BENIGN EPIDERMOLYSIS BULLOSA.

M.F. Jonkman*, M.C.J.M. de Jong*, K. Heeres*, H.H. Pas*, J.B. van der Meer*, K. Owaribe†, C.M. Niessen‡ and A. Sonnenberg‡, *Department of Dermatology, University Hospital, Groningen, the Netherlands, †Department of Molecular Biology, Nagoya University, Nagoya, Japan, and ‡Department of Cell Biology, The Netherlands Cancer Institute, Amsterdam, the Netherlands

In this study we report a patient, with non-lethal junctional epidermolysis bullosa (JEB) and alopecia: generalized atrophic benign epidermolysis bullosa (GABEB), who was found to have a deficiency of 180-kD bullous pemphigoid antigen (BP180). We applied the immunofluorescence technique on specimens of clinically normal skin of seven junctional epidermolysis bullosa (JEB) patients (4 lethal, 1 GABEB, 1 cicatricial, 1 pretibial) with moab 1D1, which binds to the extracellular domain of 180-kD bullous pemphigoid antigen (BP180), and moab 815, which binds to 230-kD bullous pemphigoid antigen (BP230) [J Biochem 1993; 113: 493-501]. No binding with moab 1D1 was observed in the GABEB specimen, whereas binding with moab 815 was normal. In the *other* six JEB specimens included in this study binding of moabs 1D1 and 815 was normal. No binding to GABEB skin was seen with polyclonal serum from a patient with bullous pemphigoid (BP), which on immunoblot only recognized the 180-kD antigen, whereas normal binding was found when "mixed type" polyclonal BP-serum, that recognizes both BP180 and BP230 in immunoblot, was used. Immunofluorescence staining with monoclonal antibodies GB3 (nicein/kalinin) and LH7:2 (collagen type VII) was normal in the skin of the GABEB patient.

The results were confirmed by immunoblot using "mixed type" BP-serum that showed that only the 230-kD antigen, and not the 180-kD antigen, is present in extracts of cultured keratinocytes of the GABEB patient.

Previous studies on the expression of bullous pemphigoid antigen(s) in JEB skin were inconclusive, since, at that time, non-specific polyclonal sera from patients with bullous pemphigoid had been used. The present study with the monoclonal antibody 1D1 has revealed the deficiency of BP180 in a patient with GABEB.

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Point mutations in the regions of the COL7A1 gene coding for the NC-1 and NC-2 domains of collagen VII in patients with recessive epidermolysis bullosa dystrophica (R-EBD).

D.U. Kalinke*, U.Kalinke**, A. Baldi*, J.-O. Winberg&, J. Lauharanta§, J.Uitto" and L. Bruckner-Tuderman*+ *Department of Dermatology and **Institute of Experimental Immunology, University Hospital, Zurich, Switzerland; &Polar Institute of Medical Genetics, Tromsö, Norway; §Department of Dermatology, University of Helsinki, Helsinki, Finland; "Jefferson Medical College, Thomas Jefferson University, Philadelphia, PA, U.S.A.; *+Department of Dermatology, University of Münster.

In the skin of patients with the mutilating or inversa type of recessive epidermolysis bullosa dystrophica (R-EBD) anchoring fibrils are rudimentary or absent, leading to severe sub-lamina densa blistering of the skin. Collagen VII, the major component of the anchoring fibrils, is the candidate gene linked to R-EBD. Immunofluorescence staining of fibroblasts from 6 patients with R-EBD mutilans and 6 patients with R-EBD inversa was faint or negative with antibodies to collagen VII. However, Northern blot analysis revealed normal size mRNA in all patients fibroblasts. Overlapping fragments, covering the globular NC-1 and NC-2 domains of COL7A1, were ampli-fied with RT-PCR. Comparable lenghts of the amplimers from patients and controls excluded major deletions or insertions responsible for the defective protein. To find point mutations the amplimers were analyzed with PCR-SSCP. In 4/6 R-EBD inversa patients and in 1/6 R-EBD mutilans patient band shifts were found in the NC-1 domain. In two unrelated patients the band shift resulted from a C-nucleotide deletion at the same position. Three of the patients with a band shift in the NC-1 domain showed an additional shift in the NC-2 domain. One patient (with C-nucleotide deletion in the NC-1 domain) had a T to C transition in the NC-2 domain, leading to an amino acid exchange from leucine to proline. It is now under investigation, whether the two mutations are located on the identical or on two different alleles.

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DETERMINATION OF HUMAN PAPILLOMA VIRUS IN NON-ANOGENITAL SQUAMOUS CELL CARCINOMAS BY POLYMERASE CHAIN REACTION.

Youn H. Kim, Lori Fukuhara Serkland, Eugene A. Bauer, Bruce Smoller, and Joel M. Palefsky, Dept. of Dermatology, Stanford Univ. School of Med., Stanford, CA, and Dept. of Stomatology, Univ. of California, San Francisco, San Francisco, CA.

The role of human papilloma virus (HPV) in tumorigenesis and/or progression of non-anogenital squamous cell carcinomas (SCCs) is controversial. Systemic or local host-defense impairment may provide a permissive milieu for HPV oncogenesis and tumor progression. Paraffin-embedded or frozen tissues of SCCs, actinic keratoses (AKs), keratoacanthomas (KAs), and atypical porokeratoses (PKs) obtained from non-anogenital sites of organ transplant, recessive dystrophic epidermolysis bullosa (RDEB), cutaneous T-cell lymphoma (CTCL), and otherwise healthy, actinically-damaged patients were probed for HPV DNA using polymerase chain reaction (PCR). Amplified products were detected and typed using dot blot and hybridization methods using HPV consensus and type-specific oligo probes (HPV 6,11,16,18,31,33,39,45,51,52). Beta-globin primers were used as positive controls for the PCR studies. One of 5 SCCs, 2 of 5 PKs, and 1 of 2 AKs obtained from organ transplant patients contained HPV DNA. Three of 3 SCCs obtained from 2 RDEB patients contained HPV DNA; HPV type of one SCC is 16 while that of the other 2 SCCs is unknown. One SCC and one KA tissues from 2 CTCL patients did not contain HPV DNA. One of 3 AKs and 1 of 3 KAs from otherwise healthy, actinically-damaged patients contained HPV DNA, both typed as 16; HPV DNA was not detected in one SCC tissue studied. The data indicate that there is no definitive pattern of HPV detection or type-specificity in clinical phenotypes of variable systemic or local host-defense status; although, all three RDEB SCCs (100%) contained HPV DNA compared with 20-30% prevalence in each organ transplant and otherwise healthy, actinically-damaged patients. Studies are in progress to further explore the mechanism(s) of epidermal carcinogenesis, specifically, the potential interactive role of tumor suppressor genes with dominant oncogenes in tumor promotion and/or progression.

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SEVERE DYSTROPHIC EPIDERMOLYSIS BULLOSA IN A BOSNIAN REFUGEE FAMILY.

D Koss-Harnes,Department of Dermatology,Rikshospitalet and University of Oslo;I Anton-Lamprecht,Department of Dermatology,Ruprecht-Karls University Heidelberg;L Bruckner-Tudermann,Department of Dermatology,Westfälische Wilhelms Universitat,Münster.T Gedde-Dahl jr,Department of Dermatology and Institute of Forensic Medicine,Rikshospitalet and University of Oslo.

A 2-year-old girl from Mostar,Bosnia,had blisters especially in shoulder/upper arm region and thigh at birth, later in areas of friction and trauma and the oral cavity.She has complete loss of nails and scarring and milia in areas of previous blistering.The family history is negative and the parents are not known to be consanguineous.A skin biopsy of clinically normal skin from the left buttock revealed no pathological changes by light microscopy.Electron microscopy showed focal areas of splitting just underneath the basal lamina and complete absence of anchoring fibils at the blister roof apart from some granular material.Unsplit areas showed severely hypoplastic anchoring fibrils.No further abnormalities were found in the connective tissue and epidermis.Immunofluorescence showed absence of type VII collagen by the use of LH-7.2,GDA-JF3 and polyclonal col.VII antibodies.DNA-typing of the family including a healthy brother is in progress.These results indicate that the patient has Epidermolysis bullosa dystrophica Hallopeau-Siemens of the severe generalized mutilating subtype which is prone to synechias.

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SURGICAL TREATMENT OF EPIDERMOLYSIS BULLOSA (RECESSIVE DYSTROPHIC) AND POSTOPERATIVE SPLINTING.

AL Ladd and A Kibele. Departments of Functional Restoration and Rehabilitation Services. Lucile Salter Packard Children's Hospital at Stanford. Stanford, CA.

Four children with Epidermolysis Bullosa (Recessive Dystrophic) age 3-14 underwent surgical release of syndactyly and hand contractures. The surgical technique included de-cocooning the hand and fingers, manipulation of contracted joints, and full thickness skin grafting to dermal defects created by release of contractures. Three of the four were performed under anesthesia of ketamine and sedation, one required general endotracheal intubation. Syndactylies and digital contractures necessitating skin grafts ranged from one to all digits, and one hand required extensive skin grafting of the palm. Two patients underwent gastrostomy tube placement concurrently. All patients were casted for two weeks in a position of wrist and digital extension with maximum web opening maintained. Cast changes were performed at two weeks in the operating room; under ketamine and sedative anesthesia. Patients underwent extensive postoperative splinting and wound care. At approximately two to four days after cast removal a thermoplastic splint was fabricated, which incorporated web retaining spacers. Silastic putty was also used for web retention.

Postoperative splints were worn 24 hours for four weeks, then at night subsequently. Minimal contractures have recurred in this short term followup (8-15 months), but appear to be highly related to compliance with splint wear, and ongoing interaction with a hand therapist knowledgeable with the disease.

In summary, we believe early and aggressive surgical release of hand contractures and judicious postoperative splinting may prevent or postpone loss of hand function in children with Epidermolysis Bullosa (Recessive Dystrophic). This requires an interdisciplinary team who enables this care and family compliance. Ongoing studies continue.

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GENERALIZED GRAVIS JUNCTIONAL EPIDERMOLYSIS BULLOSA: CLINICAL OBSERVATIONS, LABORATORY FINDINGS AND ESTABLISHMENT OF AN IMMORTALIZED KERATINOCYTE CELL LINE.

K. Lim*, M. McEvoy*, W.P.D. Su*, M.R. Pittelkow*†, Departments of Dermatology* and Biochemistry, Molecular Biology†, Mayo Foundation, Rochester, MN.

A fullterm infant is described with junctional epidermolysis bullosa (JEB). The distribution and morphologic characteristics of generalized blistering in areas of pressure and perioral and perinasal granulation tissue suggested the diagnosis of generalized gravis (Herlitz) JEB. The family history was consistent with autosomal recessive inheritance. Electron microscopy demonstrated a subepidermal cleft arising in the lamina lucida with hemidesmosomal hypoplasia consistent with gravis JEB. Immunofluorescent antigenic mapping localized laminin and Type IV collagen exclusively to the blister base and weak reactivity of bullous pemphigoid antigen to both the roof and base. Type VII collagen (LH 7:2 epitope) was detected solely at the base of the cleavage plane, and abnormal staining of kalinin (GB3, nicein) and 19-DEJ-1 antigen was observed. During initial hospitalization, an intact blister roof was removed, epidermal cells disaggregated, and keratinocytes were cultivated in complete MCDB153 medium. Primary cultures were established. The patient died at age two months of sepsis. Secondary cultures of keratinocytes were conditionally immortalized by transfection with a ts-SV40 construct using a lipofection procedure. Following growth to high density and repeated passage, the majority of cells became senescent. However, proliferative foci of keratinocytes were observed and expanded by serial subculture. The growth of subcultures has remained vigorous and cells exhibit similar properties as the primary cell cultures including spontaneous loss of adherence from the plastic substratum and ease of trypsin removal from the flask. DNA has been extracted from cultured keratinocytes for further molecular genetic analysis. It is anticipated that the cell line will be useful in further studies to characterize the cellular and molecular biological defects in this form of JEB.

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INVERSE DYSTROPHIC EPIDERMOLYSIS BULLOSA: REPORT OF TWO CASES WITH FURTHER CORRELATION BETWEEN IMMUNOFLUORESCENCE STUDIES AND ELECTRON MICROSCOPY. AN Lin*, LT Smith**, J-D Fine***.*The Rockefeller University, New York, NY. **University of Washington School of Medicine, Seattle, WA. ***University of North Carolina at Chapel Hill, NC.

We present two patients with dystrophic EB whose lesions occured in striking inverse distribution. A 17 year old boy presented 4 years ago with blisters mainly in the groin, ankyloglossia, dysphagia and microstomia which subsequently required plastic surgical reconstruction. Barium swallow showed proximal esophageal narrowing. Biopsy of a fresh blister showed separation below the lamina densa and diminished anchoring fibrils on electron microscopy, but normal staining of LH 7:2 on immunofluorescence. A 9 year old boy presented 4 years ago with blisters mainly in the axillae and groin, short frenulum and no dysphagia. Electron microscopy had previously shown separation below the lamina densa, and repeat biopsy showed sparse immature anchoring fibrils but normal staining with LH 7:2 antigen. These findings suggest that inverse dystrophic EB is a rare, unique subset characterized by normal LH 7:2 staining but diminished anchoring fibrils, variable clinical course, and marked oral and esophageal involvement in some patients. It may possibly be caused by a point mutation in the type VII collagen gene at a site whose expression is not detected by the monoclonal antibody LH 7:2.

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ANCHORING FIBRIL MORPHOLOGY AND TYPE VII COLLAGEN EXPRESSION IN DYSTROPHIC EPIDERMOLYSIS BULLOSA. J.A.McGrath, A.Ishida-Yamamoto, I.M.Leigh,[1] R.A.J.Eady. Department of Cell Pathology, St John's Institute of Dermatology, St Thomas's Hospital, London, U.K. [1]Department of Experimental Dermatology, The Royal London Hospital, London, U.K.

Dystrophic epidermolysis bullosa (DEB) is characterized by various abnormalities of anchoring fibrils, which are mainly composed of type VII collagen, at the dermal-epidermal junction. To define these changes more clearly, skin samples from 22 patients with different forms of DEB were examined by pre-embedding immunoelectron microscopy using an antibody (LH 7:2) which binds to the NC-1 globular domain of type VII collagen, followed by 1nm colloidal gold-labelled secondary antibodies and subsequent silver enhancement. In dominant DEB cases, there was only a slight but variable reduction in the immunolabelling density on anchoring fibrils and on the lamina densa, in parts similar to normal human skin. In localized recessive DEB skin, some fibrillar structures just below the lamina densa (and particularly subjacent to hemidesmosomes) had specific antibody labelling despite their lack of resemblance to definitive anchoring fibrils. Immunolabelling with LH 7:2 was also seen within basal keratinocyte endoplasmic reticulum and cytoplasmic vesicles in some DEB patients, usually with milder phenotypic features. Even in the most severe cases of generalized recessive DEB, occasional immunolabelling was found within the lamina densa and on scanty thin filamentous structures at sub-lamina densa sites usually occupied by anchoring fibrils. This study suggests that DEB patients express some type VII collagen NC-1 domain epitopes that may be variably reduced at the dermal-epidermal junction or retained within basal keratinocytes. The clinical heterogeneity in DEB is mirrored by a range of immunoelectron microscopy findings, indicating variability in completeness of anchoring fibril formation and a possible spectrum of underlying type VII collagen structural protein abnormalities.

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FIBRILLIN IMMUNOREACTIVITY AT THE DERMAL-EPIDERMAL JUNCTION IN DYSTROPHIC EPIDERMOLYSIS BULLOSA. J.A.McGrath, L.Y.Sakai,[1] R.A.J.Eady. Department of Cell Pathology, St John's Institute of Dermatology, St Thomas's Hospital, London, U.K. [1]Shriner's Hospital Research, Portland, Oregon, U.S.A.

The structural composition of fibrillar structures present beneath the lamina densa in skin from patients with recessive dystrophic epidermolysis bullosa (RDEB) was studied by indirect immunofluorescence and by pre-embedding immunogold electron microscopy using a monoclonal antibody to fibrillin, a glycoprotein component of elastic microfibrils. Samples from twelve cases of generalized RDEB, six cases of the localized form of the disorder, and eight normal controls were examined. Skin from a 15 week fetus affected with generalized RDEB was also assessed. In all post-natal skin samples, immunogold fibrillin labelling was present on a lattice-like arrangement of fibrils extending perpendicularly from the lamina densa into the upper dermis. In intact (non-blistered) RDEB samples there was no difference from the control skin in either the quantity of the labelling or in the ultrastructural appearances of the immunolabelled fibrils. However, a sub-lamina densa level of blistering was present in many of the RDEB samples. Inserting into the lamina densa in such sites were a number of thin, short, fragmented wisp-like structures that still labelled for fibrillin despite a lack of ultrastructural resemblance to normal elastic microfibril bundles. Similar wisp-like structure immunolabelling was also seen in the fetal skin sample. This study suggests that as a consequence of blistering in RDEB, elastic microfibril bundles at the dermal-epidermal junction are disrupted and fibrillin-containing fibrils may be present as irregular wisp-like structures in the blister roofs. These structures may resemble rudimentary anchoring fibrils. Elastic fibrils may contribute towards securing adhesion between the epidermis and the dermis.

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SQUAMOUS CELL CARCINOMAS IN DYSTROPHIC EPIDERMOLYSIS BULLOSA. J.A.McGrath, O.M.V.Schofield, P.H.McKee, R.A.J.Eady. Department of Cell Pathology, St John's Institute of Dermatology, St Thomas's Hospital, London, U.K. Department of Histopathology, St Thomas's Hospital, London, U.K.

Several forms of epidermolysis bullosa (EB) have been reported in association with cutaneous malignancy. In this study, the clinicopathological features of 10 EB patients with this complication are presented. Eight generalized recessive dystrophic EB patients, one case of dominant dystrophic EB, and one patient with non-lethal junctional EB, aged 24-55 years with a total of 29 squamous cell carcinomas were reviewed. Two patients died from metastatic disease associated with invasive poorly differentiated squamous cell carcinomas. Five cases had multiple primary squamous cell carcinomas, including three patients with simultaneous multifocal disease. Twenty-eight of the 29 squamous cell carcinomas arose on the limbs. Histology revealed that most of the squamous cell carcinomas were well or moderately differentiated (22/29). Unusual histological findings included two verrucous squamous cell carcinomas as well as a spindle cell (angiosarcoma-like) squamous cell carcinoma. Immunohistochemical labelling with an antibody to the p53 tumour suppressor gene was positive in only six of 23 tumours studied. p53 labelling was more frequently seen in less well differentiated squamous cell carcinomas and was usually associated with a more aggressive biological behaviour of the tumour. Only one well differentiated squamous cell carcinoma (1/16) had positive p53 staining, and that was confined to the advancing border of the neoplasm. Most of the squamous cell carcinomas developed in areas of chronic non-healing ulceration (10/29) or longstanding hyperkeratotic crusting (14/29). The dermis around or beneath the carcinomas was densely scarred, more so than in non-malignant areas. In some cases it was difficult to distinguish the clinical appearances of certain areas of chronic ulceration, scarring and crusting typical of dystrophic EB from many of the squamous cell carcinomas. This study underlines the need for constant vigilance for the development of carcinomas in this group of patients, the occasional diagnostic difficulty, and the potential for metastasis.

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FEEDING TUBE GASTROSTOMY. Joseph S. McGuire, Sheila Gibbons, Lexie Nall, and Eugene A. Bauer. Department of Dermatology, Stanford University, Stanford, CA.

Nutrition is a problem in patients who suffer from types of epidermolysis bullosa (EB) involving the oropharynx and esophagus. Chronic erosions and infection of the skin create an increased demand for calories, which is met by foods of high caloric value. When this fails, two surgical applications are considered: colonic transposition and feeding tube gastrostomy.

The use of the feeding tube implantation in 16 patients is being evaluated in the Stanford University Southwestern Clinical Center of the National Epidermolysis Bullosa Registry. Of this study population, 10 patients had their initial examination and gastrostomy at Stanford; six, at other facilities. Feeding tube gastrostomy is an evolving technique, which has been utilized since the mid-1980s to improve nutrition in cystic fibrosis and cerebral palsy patients. Currently it may represent a promising strategy to enhance nutrition in EB patients, the majority of whom fall within the 5 - 10 percentile for height and weight. Although our work is still in progress, we are gathering detailed data on the 10 Stanford patients, who have had enteral supplementation through feeding tube gastrostomy. The parameters of our data collection include demographics, medical history (emphasizing gastrointestinal difficulties, e.g., dysphagia, esophageal strictures), surgical intervention (e.g., esophageal dilatation, feeding tube gastrostomy) as well as maintaining a visual analog of behavioral factors (e.g., parent-child interaction, mainstreaming, self-esteem). A control group is being drawn to match the study population on sex, race, type of EB, age-of-onset, but without tube gastrostomy. The same data gathering parameters of the study population will be applied to the controls for comparative study.

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EXPRESSION OF TYPE VII COLLAGEN IN HUMAN FETAL SKIN. J. R. McMillan and R. A. J Eady, Department of Cell Pathology, St John's Institute of Dermatology, St Thomas's Hospital, London, U.K.

Type VII collagen is the major component of anchoring fibrils. During ontogeny both basal keratinocytes and fibroblasts have been shown to synthesize type VII collagen as early as 8-9 weeks estimated gestational age (EGA). However, little is known about the precise mechanisms involved in the synthesis and secretion of type VII collagen into the extracellular space and its assembly into anchoring fibrils. We assessed the ultrastructural localization of type VII collagen using the LH7:2 antibody in samples of developing embryonic (n=4), fetal (n=4) and postnatal (n=4) digit skin by indirect immunofluorescence (IIF), pre- and post-embedding immunogold (5nm) electron microscopy (IEM). Before 8 weeks' EGA no fluorescence was detectable but by 9 weeks' EGA there was punctate labelling at the dermal-epidermal junction (DEJ). IEM showed labelling localized to beneath early hemidesmosomes but not associated with definitive anchoring fibrils. At 9 weeks IIF staining was present at the lateral surfaces of basal cells although staining was more intense along the basal pole of the cell. Staining was noted around fibroblasts in close proximity to areas of possibly reduced DEJ staining. IEM labelling was present both inside and outside dermal fibroblasts. Labelling was also present inside the basal cell cytoplasm and in the intercellular space. At 15 weeks' IIF showed linear DEJ staining which by IEM was localized to anchoring fibrils. Intracellular labelling was reduced. In postnatal skin staining at the DEJ was brighter and IEM labelling was present on the ends of cross banded fan-like anchoring fibrils. This study shows that during fetal development type VII collagen is expressed in both keratinocytes and fibroblasts. It then becomes incorporated into rudimentary anchoring fibrils under hemidesmosomes. Similar changes have been observed during wound healing.

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IDENTIFICATION OF A HOMOZYGOUS EXON SKIPPING MUTATION IN THE LAMB3 GENE IN A PATIENT WITH HERLITZ JUNCTIONAL EPIDERMOLYSIS BULLOSA. G. Meneguzzi[1], J. Vailly[1], L. Pulkkinen[2], A. Christiano[2], C. Baudoin[1], C. Miquel[1], D. Gerecke[3], R. Burgeson[3], J. Uitto[2] and J.-P. Ortonne[1], [1]U385 INSERM, Fac. de Médecine, Nice, France, [2]Dept of Dermatology, Thomas Jefferson University, Philadelphia, PA, [3]Dept of Dermatology, Harvard Medical School, Charleston, MA.

Previous studies correlated the Herlitz's junctional epidermolysis bullosa (H-JEB) to an altered detection of the basement membrane protein nicein/kalinin, recently renamed laminin-5. Expression of the three chains of laminin-5 in 6 H-JEB patients from 5 distinct kindreds was studied by immunofluorescence on skin biopsies, immunoprecipitation, and northern blot analysis of extracts from cultured H-JEB keratinocytes, using polyclonal antibodies raised against each individual chain and cDNA probes for each polypeptide. In two patients, the disease correlated with an impaired synthesis of laminin $\gamma 2$ chain, in three others with that of laminin $\beta 3$ chain and in the sixth patient with that of laminin $\alpha 3$ chain. In one kindred, analysis of the 3.8 kb cDNA coding for laminin $\beta 3$ chain revealed presence of an homozygous mutation consisting in a point deletion at position 1697 that leads to a frameshift of the open reading frame and to a premature stop codon. Northern blot analysis of cultured H-JEB keratinocytes showed that the presence of the premature stop codon was associated with an instability of the corresponding mRNA. The distribution of the mutated allele in the members of this family confirmed the direct implication of the mutation in the pathology. These results suggest that all the three chains ($\alpha 3$, $\beta 3$, $\gamma 2$) of laminin-5 are subject to mutations leading to the H-JEB phenotype.

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A RECURRENT PREMATURE TERMINATION CODON MUTATION (498insA) IN THE TYPE VII COLLAGEN GENE (COL7A1) IN TWO UNRELATED FAMILIES WITH RECESSIVE DYSTROPHIC EPIDERMOLYSIS BULLOSA (RDEB) IN ITALY. Alessandra Morricone, Angela M. Christiano, Mauro Paradisi, Corrado Angelo, Rino Cavalieri and Jouni Uitto Jefferson Medical College, Philadelphia PA, and Istituto Dermatopatico Dell'Immacolata, Rome, Italy.

Epidermolysis bullosa (EB) is a group of genodermatoses characterized by blister formation in response to mechanical trauma. In the most severe, dystrophic (scarring) forms of EB, blisters form below the cutaneous basement membrane zone, at the level of the anchoring fibrils. Ultrastructural studies of altered anchoring fibrils and genetic linkage to the gene encoding type VII collagen (COL7A1) have implicated COL7A1 as the candidate gene in the dystrophic forms of EB. We have recently cloned the gene and cDNA for type VII collagen, and have successfully identified several mutations involved in recessive dystrophic EB (RDEB). In every case analyzed thus far, the mutations resulting in the Hallopeau-Siemens type of RDEB have been premature termination codons in the COL7A1 gene. We recently studied eight families with RDEB from central and southern Italy, and found a 1 bp insertion in exon 4 of the COL7A1 gene (498insA) in two unrelated families. One patient is from the region of Abruzzi, while the other is from the region of Calabria, about 400 miles away. The families are not known to be distantly related, and both patients are compound heterozygotes for this mutation and another as yet unknown mutation(s). The parents of the patient in Abruzzi are second cousins, yet the mutation 498insA is transmitted only on the paternal allele. This mutation has not been found in a panel of 73 unrelated DEB patients from around the world, indicating that it may have originated in the Italian gene pool long ago.

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ASSESSMENT OF POSSIBLE ASSOCIATION BETWEEN PATIENTS WITH EBS HERPETIFORMIS (DOWLING-MEARA VARIANT) AND TERATOGENIC/MUTOGENIC AGENTS: APPLICATION OF CLINICAL, BIOCHEMICAL, AND GENETIC STRATEGIES. Lexie Nall, Sheila Gibbons, Joseph S. McGruire, Eugene A. Bauer. Department of Dermatology, Stanford University, Stanford, CA.

The Dowling-Meara (D-M) variant of Epidermolysis Bullosa Simplex (EBS) is transmitted as an autosomal dominant trait, presenting at birth or in early infancy with severe widespread blisters and erosions of the skin and oral mucosa. It usually follows a relatively benign course and many individuals improve in later childhood or in adult life. Histologically, there is a highly characteristic cytoskeletal abnormality, namely, aggregation or clumping of tonofilaments which is not seen in any other form of EBS.

Since the late 1950s, it is estimated that more than 750 million tons of toxic chemical wastes have been discarded in 30,000 to 50,000 hazardous waste sites in the United States. It is the objective of this investigation to initiate a pilot study to assess the possible association between patients with EBS/D-M and teratogenic/mutagenic chemical and physical agents found in hazardous waste. In the Stanford University Southwestern Clinical Site of the National Epidermolysis Bullosa Registry, 22 patients have biopsy-proven D-M . In an effort to determine the genetic impact of potential exposure to toxic agents, the parents of the D-M patients were asked to respond to a self-administered questionnaire containing such variables as geographic residence, occupational history, exposure to environmental chemical and physical agents, use of medications, and recreational drugs. The time frame was set at approximately 10 years prior to the conception of their affected child. Responses were tabulated and computerized pedigrees drawn.

Although the questionnaire findings did not yield a positive correlation between environmental factors and the diagnosis of the D-M variant, there was sufficient evidence to lead us to pursue (in an expanded NEBR sample) the potential harmful effects of toxins in precipitating mutations in D-M patients.

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KERATIN GENE MOSAICISM IS THE GENETIC BASIS FOR EPIDERMAL NEVUS, EPIDERMOLYTIC HYPERKERATOSIS TYPE. AS Paller, AJ Syder, Y-M Chan, Q-C Yu, E Hutton, G Tadini and E Fuchs. Departments of Pediatrics and Dermatology, Northwestern University, Departments of Molecular Genetics and Cell Biology, University of Chicago, Chicago, IL, and Department of Dermatology, University of Milan, Milan, Italy.

Recent studies have linked keratin gene abnormalities to two autosomal dominant blistering disorders, epidermolytic hyperkeratosis (EH) and epidermolysis bullosa of the simplex type. Epidermal nevi are considered a form of somatic mosaicism, and tend to follow Blaschko's lines in their distribution. Epidermal nevi of the EH type demonstrate ultrastructural changes similar to those of EH, with perinuclear clumping of keratin tonofilaments and epidermolysis in suprabasal layers. Furthermore, offspring of patients with epidermal nevi, EH type may have generalized EH. These observations suggest that spontaneous somatic mutations in keratins 1 and 10 result in epidermal nevi. To explore this possibility, we obtained biopsies of lesional and nonlesional skin for mRNA extraction from cultured keratinocytes, for genomic DNA extraction from fibroblasts, and for electron microscopy of patients with epidermal nevus, EH type. Genomic and cDNAs were analyzed by sequence analysis and restriction enzyme digests. Our findings provide convincing evidence that some forms of epidermal nevi occur as a result of spontaneous somatic mutations in keratins K1/10. Furthermore, these studies of patients with clinical, ultrastructural and genetic mosaicism for K10 gene defects provide the most definitive evidence to date that, in humans, gene abnormalities in K1/10 cause EH.

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MUTATIONS IN THE LAMININ 5 GENES (LAMB3 AND LAMC2) IN PATIENTS WITH THE JUNCTIONAL FORMS OF EPIDERMOLYSIS BULLOSA (JEB). Leena Pulkkinen, Angela M. Christiano, Donald Gerecke*, Karl Tryggvason^, Robert E. Burgeson* and Jouni Uitto Jefferson Medical College, Philadelphia, PA, *Cutaneous Biology Research Center, Boston, MA, and ^University of Oulu, Finland.

Junctional epidermolysis bullosa (JEB) is an autosomal recessive disorder characterized by blister formation within the dermal-epidermal basement membrane zone. On the basis of previous immunofluorescence studies using anti-kalinin/laminin 5 antibodies which showed an absence of staining in the skin of JEB patients, the three genes for the laminin 5 subunits became candidate genes for mutations in JEB. Using RT-PCR and heteroduplex analysis, we have characterized two distinct mutations in the LAMC2 gene (previously known as kalinin B2) and one mutation in the LAMB3 gene (previously known as kalinin B1) in three patients with JEB. The first case was a 23-year old female with JEB who was found to be homozygous for a G-to-A substitution at the 3' acceptor splice site of intron 8 of the LAMC2 gene, resulting in the in-frame skipping of exon 9 from the LAMC2 mRNA. In a second case, a 10-year old male with JEB was found to be heterozygous for a 20bp deletion/1 bp insertion in the LAMC2 gene, which results in a frameshift and premature termination codon on one allele. In a third case, a 22-year old male was found to be heterozygous for a 1 bp deletion in the LAMB3 gene, resulting in a frameshift and premature termination codon on one allele. In these two patients, the second mutations are as yet unknown. Collectively, these findings demonstrate that mutations in the LAMC2 and LAMB3 genes are the underlying cause of some forms of JEB. Understanding the molecular defects in patients with JEB will form the basis for DNA-based prenatal diagnosis and gene replacement therapy for these patients in the future.

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CONFOCAL MICROSCOPIC ANALYSIS OF THE DISTRIBUTION OF TWO SKIN BASEMENT MEMBRANE COMPONENTS. Kathryn Riddelle, Anthony Daniels, Li Zeng, and Jo-David Fine, Department of Dermatology, University of North Carolina at Chapel Hill, Chapel Hill, NC.

The study of epithelial cell adhesion to underlying matrices has yielded the discovery of several new proteins localizing to the basement membrane zone. Two such proteins, nicein (kalinin; epiligrin) and uncein, are known to reside primarily within the lowermost and uppermost lamina lucida, respectively. Immunoelectron microscopy data suggest that both are associated with anchoring filaments. Using confocal immunofluorescence microscopy, the spatial distribution of uncein and nicein were compared and contrasted to the distribution of other cytoskeletal components. Monolayers of normal human keratinocytes were stained with either 19-DEJ-1 (anti-uncein) or GB3 (anti-nicein) monclonal antibodies, used in combination with polyclonal antibodies directed against keratin intermediate filaments, the $\alpha 6$ integrin, the $\alpha 3$ integrin, actin, vinculin, and bullous pemphigoid antibodies (the latter a marker of hemidesmosomes). Anti-nicein antibodies stained intercellular regions intensely, with more diffuse staining observed underneath cells. In distinct contrast, anti-uncein antibodies generated staining underneath cells but noticeably absent from intercellular regions. Anti-uncein antibodies, but not anti-nicein antibodies, co-localized with both bullous pemphigoid sera and $\alpha 6$ integrin at the cell-substratum interface. There was no obvious co-localization of the cytoskeletal networks of keratin intermediate filaments or actin microfilaments with either uncein or nicein, although the possibility of indirect interactions still exists. The staining observed with anti-uncein was different from that generated by anti-vinculin and anti-integrin $\alpha 3$ antibodies. This would seem to imply that uncein is associated with hemidesmosomes and is excluded from focal adhesions. In addition, these data further suggest that uncein and nicein are distinct proteins.

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A 1bp DELETION (5818delC) IN EXON 70 OF THE TYPE VII COLLAGEN GENE IN A JAPANESE PATIENT WITH RECESSIVE DYSTROPHIC EPIDERMOLYSIS BULLOSA.

Hiroshi Shimizu, Angela M. Christiano, Jouni Uitto, Atsuko Tanaka and Takeji Nishikawa.

The Hallopeau-Siemens type of recessive dystrophic epidermolysis bullosa (RDEB) is a life-threatening genodermatosis characterized by loss of dermal-epidermal adherence with abnormal anchoring fibrils. A 6-month-old Japanese boy presented with typical clinical features of the Hallopeau-Siemens type of RDEB with severe fusion of toes. Electron microscopy of the blister showed the separation just beneath the lamina densa, and demonstrated no mature anchoring fibrils. Despite the severe clinical features, indirect immunofluorescence revealed the normal expression of LH7.2 antigen, as well as GB3 antigen (BM600), bullous pemphigoid antigen and type IV collagen at the basement membrane zone of the patient's skin.

Molecular analysis of the gene in this family disclosed that the patient and his father are heterozygous for a 1bp deletion of a C in exon 70 of type VII collagen, which is part of the first amino acid of the"hinge" region of the protein. This deletion (5818delC), first found in this family, causes a frameshift and premature termination codon (TGA) 64 amino acids downstream in exon 73 of the gene. Although the second mutation in this family has not yet been identified, this is the first Japanese RDEB patient in whom the pathological mutation in the type VII collagen gene was confirmed.

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DIMUNITION OF AIRWAY AND INTERTRIGINOUS GRANULATION TISSUE AFTER TOPICAL AND INTRA-LESIONAL CORTICOSTEROIDS IN AN INFANT WITH JUNCTIONAL EPIDERMOLYSIS BULLOSA (HERLITZ SUBTYPE). MK Spraker*, NW Todd**, PA O'Shea***, Department of Dermatology,* Division of Otolaryngology,** Department of Pathology,*** Emory U. Sch of Med, Atlanta, Ga.

Patients with the Herlitz variant of junctional epidermolysis bullosa (EB) usually die during infancy from airway compromise, or complications thereof, caused by bullae, erosions, and excessive granulation tissue that narrows the trachea, causing worsening respiratory distress. We have cared for 2 siblings with documented junctional EB who died during infancy. Because postmortem examination of the airway of the older sibling showed hypertrophic granulation tissue in the trachea that left an airway only 3 mm in diameter, treatment of similar granulation tissue was attempted in the younger sibling.

Topical clobetasol propionate (Temovate^{R}) successfully flattened hypertrophic granulation tissue that developed in intertriginous areas and around a gastrostomy site. When bronchoscopy through the tracheotomy at age 10 months revealed thickened tough seemingly mature granulation tissue at the tip of the tracheotomy tube that compromised the lumen, it was focally injected with triamcinolone acetonide (0.05 ml of Kenalog^{R} 10 mg/ml). At repeat bronchoscopy 5 weeks later, the mass had resolved, but another had arisen where the tip of the tracheotomy tube now touched. The patient died of suspected but unproven sepsis at the age of 13 months. Postmortem examination revealed diffuse obstructing intraluminal proliferations of granulation tissue within the larynx, trachea and mainstem bronchi and fixed tenacious mucous.

The presence of airway granulation tissue extending down to the bronchi, which has also been reported by others, suggests it is important to understand its pathogenesis in order to manage these patients. The response to topical and intra-lesional corticosteroids is encouraging but did not prevent the patient's demise, and historically systemic corticosteroids do not prevent worsening airway disease. Perhaps more aggressive therapy with topical corticosteroids or even α-interferon would be helpful.

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MULTIFOCAL SUPERFICIAL SPREADING MELANOMA IN A CHILD WITH RECESSIVE DYSTROPHIC EPIDERMOLYSIS BULLOSA. MK Spraker and AR Solomon. Department of Dermatology, Emory University School of Medicine, Atlanta, Ga.

Squamous cell carcinoma is a not uncommon complication in patients with dystrophic epidermolysis bullosa, and basal cell carcinoma, osteosarcoma and extramammary Paget's disease have also occurred. However, there is only one previous report of melanoma - a nodular melanoma in a 32 year old male with recessive dystrophic epidermolysis bullosa (RDEB).

We report an 11 year old child with RDEB who developed a 1.5 cm round hyperpigmented macular lesion on his left cheek. Several months later, a similar lesion developed on his right shoulder. Histologically, both lesions were superficial spreading melanoma (Breslow thickness 0.51 mm and 0.60 mm respectively, Clark's level II) radial growth phase. Both lesions were excised and to date, one year later, there has been no recurrence or metastasis.

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AN EPIDERMOLYSIS BULLOSA SIMPLEX PATIENT WITH A KERATIN 5 HOMOZYGOUS DEFECT. K. Stephens, A. Zlotogorski, L. Smith, P. Ehrlich, R. Le, E. Wijsman, V.P. Sybert. University of Washington, Seattle WA and Hadassah University Hospital, Jerusalem.

Epidermolysis bullosa simplex (EBS) is a skin blistering disorder caused by abnormal keratin filament assembly due to a mutation in either the keratin K5 or K14 gene. To determine which keratin gene was mutated in a large family with multiple consanguinous marriages, linkage analysis was performed. Significant evidence in favor of linkage between EBS and D12S14, a locus near the K5 gene, was obtained (LOD=7.60, theta =0). The K5 gene was sequenced and a codon 173 Lys-->Asn substitution identified that occurred in 33 affected family members, but not in 5 unaffected members or 25 unrelated, unaffected individuals. Linkage and sequence analysis verified that 1 affected child, from a marriage between affected 1st cousins, inherited a mutated K5 gene from both parents. In this family, clinical examination and EM of skin biopsies were consistent with the Koebner-EBS subtype. The clinical and ultrastructural phenotypes of the homozygote did not differ significantly from those of heterozygous relatives. Despite absence of any normal K5 protein in the homozygote the keratin filaments did not clump. This K5 defect is a true dominant mutation.

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ALTERED EXPRESSION OF A NEW ANTIGEN OF THE DERMAL-EPIDERMAL JUNCTION (NU-T2 DEJ Ag) IN JUNCTIONAL EPIDERMOLYSIS BULLOSA.

G Tadini, J Kanitakis°, R Cavalli, S Cambiaghi, D Schmitt°, E Berti.
Institute of Dermatological Sciences, University of Milan, Italy
° INSERM U346/Dermatopathology Laboratory, Hospital Herriot, Lyon, France.

NU-T2 Ag is a new and recently described antigen of the dermal-epidermal junction (DEJ), recognized by an anti-CD1b monoclonal antibody, namely NU-T2. This antigen is epithelial and primate-specific, and its expression is confined to the lower lamina lucida of epithelial basement membranes, and to the uppermost part of the pilosebaceous unit and sweat glands. The expression of NU-T2 Ag was studied in junctional (12 patients) and other types of hereditary epidermolysis bullosa (EB). NU-T2 DEJ Ag was completely absent both in bullous and uncleaved skin of JEB gravis. In JEB mitis, the expression of the NU-T2 DEJ Ag was variable, being absent or reduced. The expression of this antigen compared to that of GB3, suggested that these two antigens are different. Out of six patients with JEB mitis, the NU-T2 DEJ Ag was absent in five in bullous skin, and in three in uncleaved skin, while GB3 was absent in three cases in bullous skin, and always expressed in uncleaved skin. Therefore, it appears that the NU-T2 antibody is a more sensitive probe than GB3 for the diagnosis of JEB mitis. In patients suffering from simplex and dominant dystrophic forms of EB, the use of NU-T2 antibody showed normal labelling, while in some recessive forms of dystrophic EB, the expression of NU-T2 Ag was reduced in bullous skin. The results of our study suggest that NU-T2 is a novel Ag of the DEJ that seems to be important for dermal-epidermal cohesion. Although the precise molecular characterization of the NU-T2 DEJ Ag remains to be performed, we believe that the NU-T2 monoclonal antibody is a new relevant tool for the diagnosis, the prenatal diagnosis, and the classification of inherited EB, especially for those due to a lucidolitic cleavage. A genetic defect of NU-T2 DEJ Ag could be involved in the pathogenesis of JEB.

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EPIDEMIOLOGICAL SURVEY ON EPIDERMOLYSIS BULLOSA IN ITALY: FORMATION OF A NATIONAL REGISTRY.

G Tadini, L Naldi, L Locati, S Camozzi, T Cainelli
GISED and Institute of Dermatological Sciences, University of Milan, Italy.

A two year nationwide retrospective study on Hereditary Epidermolysis Bullosa (EB) was performed in our country to better define the real relevance of this group of genodermatosis. This study was undertaken in cooperation with GISED (Gruppo Italiano Studi Epidemiologici in Dermatologia) and ANEB (Associazione Nazionale Epidermolisi Bollose), involving both dermatology and neonatology departments. The study gave a complexive result of 301 patients, with an estimated prevalence of 1: 186368 (in a population of 57,000,000). Sex ratio was 52% males and 48% females. The representation of the different subtypes of EB is the following: Simplex 115 patients (generalized 62, localized 35, Dowling-Meara 17, other 1), Junctional 18 (gravis 7, mitis 9, with pyloric atresia 1, inversa 1), Dystrophic 142 (dominant 84, recessive 58), unclassified 26. Other 70 cases from all over the country are known, but not classified in the present survey. The foundation of a National Registry is mandatory in order to obtain epidemiological data, to establish Centers for diagnosis, prenatal diagnosis, treatment, and classification of the EB. Other aims are to facilitate contacts between affected families and medical staffs, and to provide social supports to the patients. A research program in EB is under project, and will be organized in cooperation with italian and foreign laboratories.

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HETEROGENEITY OF MUTATIONS IN THE TYPE VII COLLAGEN GENE (COL7A1) IN DIFFERENT FORMS OF DYSTROPHIC EPIDERMOLYSIS BULLOSA

Jouni Uitto, Alain Hovnanian* and Angela M. Christiano
Jefferson Medical College, Philadelphia, PA, and *INSERM, Paris, France.

The spectrum of clinical severity and inheritance patterns in the different forms of dystrophic epidermolysis bullosa appear to reflect different types and combinations of mutations within the type VII collagen gene. We recently reported a homozygous missense mutation (M2798K) in two siblings with the mild (mitis) form of RDEB. This mutation is thought to interfere with anchoring fibril assembly, resulting in a mild phenotype in affected individuals, while heterozygous carriers were clinically normal. In contrast, in three Hallopeau-Siemens RDEB patients with premature termination codons in the homozygous state, the consequences are profound: two premature termination codons leads to the absence of any full-length type VII collagen, and as a result, no detectable anchoring fibrils. We have observed heterozygous PTCs in fourteen additional HS-RDEB patients, and we predict their second mutation will also be a PTC. Interestingly, we have recently observed PTCs on one allele of two patients with the mitis form of RDEB, suggesting that in these patients, the second mutation must contribute to the striking difference in phenotype. We predict that the second mutation in these patients will be a subtle mutation, such as M2798K, which interferes with assembly. Furthermore, in a DDEB family, we recently identified a glycine substitution in the triple-helical region of COL7A1, which exerts a dominant/negative effect on the formation of anchoring fibrils. As additional mutations in COL7A1 in DEB are characterized, we can begin to classify, diagnose and eventually treat patients on the basis of their molecular defects.

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MUTATIONS IN THE γ2 CHAIN GENE (LAMC2) OF LAMININ-5 (NICEIN/KALININ) IN PATIENTS WITH HERLITZ'S JUNCTIONAL EPIDERMOLYSIS BULLOSA. J. Vailly[1], L. Pulkkinen[2], F. Galliano[1], A. Christiano[2], D. Aberdam[1], J. Bonifas[3], K. Tryggvason[4], E. Epstein[3], J. Uitto[2], J.-P. Ortonne[1] and G. Meneguzzi[1], [1]U385 INSERM, Faculté de Médecine, Nice, France, [2]Dept of Dermatology, Thomas Jefferson University, Philadelphia, PA, [3]Dept of Dermatology, University of California, San Francisco, CA, [4]Dept of Biochemistry, University of Oulu, Oulu, Finland.

We previously reported that expression of laminin-5 (nicein/kalinin) is specifically impaired in patients affected by Herlitz junctional epidermolysis bullosa (H-JEB). This autosomal, recessive genodermatosis is characterized by a tissue separation within the lamina lucida of the epidermal basement membrane with a cleavage plane lying below the basal keratinocytes. In four families, we have identified linkage of the Herlitz's syndrome to the gene LAMC2 coding for the laminin γ2 chain. A maximum two-point lod score of 5.33 (at θ = 0) was observed between a (CA/GT)24 microsatellite on chromosome 1q25-31 associated to this gene and the disease. In two consanguineous families, we have identified homozygous mutations in the 5' region of the cDNA for the γ2 chain. In the first family, the mutation is a transition (C-to-T) leading to a premature stop codon, in the second family it consists of a base substitution changing the donor site of the intron 7 and leading to an exon skipping with a frameshift. The distribution of the wild type and the mutated γ2 chain alleles in the members of each family is consistent with a direct implication of these mutations in the pathology and it confirms the haplotypes of the healthy carriers, as determined by linkage analyses.

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A MULTINATIONAL STUDY OF HLA ASSOCIATIONS IN AUTOIMMUNITY TO TYPE VII COLLAGEN IN PATIENTS WITH EPIDERMOLYSIS BULLOSA ACQUISITA.

EA Welsh[1], C Prost[2], V Lepage[3], D Charron[3], HO McDevitt[4], and DT Woodley[5]. Depts of Derm[1], and Microbiol & Immunol[1,4], Stanford Univ Sch of Med, Stanford, CA USA; Dept of Derm[2] and Laboratory of Immunol & Histocompatibility[3], Hôpital St. Louis, Paris, France; Dept of Derm[5], Northwestern Univ Med Sch, Chicago IL USA.

Epidermolysis bullosa acquisita (EBA) is characterized by circulating and/or tissue-bound IgG autoantibodies to anchoring fibril type VII collagen in the basement membrane zone. Two clinical presentations of EBA have been reported. The classical or non-inflammatory form (cEBA) and a generalized, inflammatory form (iEBA). Autoimmunity to type VII collagen has been reported to be associated with HLA-DR2 in black and white EBA patients in the U.S. Our previous serologic studies could not confirm this finding in white EBA patients. Instead, we found evidence for the association of other HLA haplotypes and autoimmunity to type VII collagen. The purpose of this study was to clarify these results by an analysis of the frequency of DRB1 and DQB1 alleles in a large cohort of EBA patients (n=25, all of Caucasian European decent). We determined the DRB1 and DQB1 genes using sequence-specific oligonucleotide probes hybridized to amplified genomic DNA from 8 white patients from France with cEBA and 17 white EBA patients (6 known cEBA) from the U.S. In all cEBA patients analyzed, we observed an increased frequency of DRB1*0101/02 (36%), and *0301 (31%) versus 21% and 18% in controls, respectively, and a smaller increase in DRB1*08 (14% vs. 7% in controls). Analysis of the DQB1 alleles resulted in an increase in the frequency of the DQB1*0501 allele in cEBA patients (46% vs. 22% in controls). When all EBA patients were analyzed together, a more pronounced increase in the frequency of DRB1*08 (24% vs 7%) was observed. An increase in the frequency of DQB1*0501 (38% vs. 22%) was still observed. In this study the frequencies of the DR2 alleles, DRB1*1501 and *1502, were not significantly different from control values in any EBA cohort analyzed. These results suggest that DRB1*0101/02, *0301, and DQB1*0501 may be important risk factors associated with autoimmunity to type VII collagen in cEBA. Additionally, different HLA haplotypes may be involved in susceptibility to these two clinically different forms of EBA. These data are consistent with our hypothesis that the pathogenesis of EBA may involve an antigenic trigger within the setting of a susceptible HLA environment.

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RELATION OF ANCHORING FIBRIL STRUCTURE, COLLAGEN VII AND MATRIX METALLOPROTEASE EXPRESSION IN RECESSIVE DYSTROPHIC EPIDERMOLYSIS BULLOSA.

JO Winberg[1], I Anton-Lamprecht[2], A König[3], Ø Nilssen[4], T Gedde-Dahl Jr[5] and L Bruckner-Tuderman[6]. [1]Biochemistry Department, IMB, University of Tromsø, [2]Institute for Ultrastructural Research of the Skin, University of Heidelberg, [3]Department of Dermatology, University Hospital of Zürich, [4]Department of Medical Genetics, University Hospital of Tromsø, [5]Institute of Forensic Medicine and Department of Dermatology, Rikshospitalet and University of Oslo and [6]Department of Dermatology, University of Münster.

Four patients with severe mutilating recessive dystrophic epidermolysis bullosa (REBD-M), one with generalised non-mutilating REBD and one patient with REBD inversa have been investigated with respect to the structure of anchoring fibrils (AF), presence of collagen VII, the major AF protein and expression of neutral matrix metalloproteases (MMP) and their inhibitors. Two of the REBD-M patients lacked AF and collagen VII in non-blistered skin, while the other two were more unusual. The non-mutilating, the inversa patients and one REBD-M patient stained positive for collagen VII and showed normal amount of normal appearing AF in non-blistered skin, but no AF in blistered skin. These AF/collagen VII positive patients and one of the AF/collagen VII negative patients showed a normal amount of MMP activity in non-blistered skin, while in blistered skin a significant elevation of the MMP activity was seen. The fourth REBD-M patient showed a weak positive staining with collagen VII antibodies and exhibited some normal AF, but most of the fibrils were of variable length, some showed disintegration of the outer segment and some had only half the normal length. The unaffected skin of this patient revealed an elevated level of MMP activity, which may explain the ultrastructural findings. However, it appears that matrix-cell interactions may be important in the pathogenetic events, as linkage studies indicate that the collagen VII gene is identical with the REBD gene. Taken together, our results suggest heterogeneous causative pathogenetic mechanisms behind REBD.

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THE ENAMEL ULTRASTRUCTURE OF TEETH IN HEREDITARY EPIDERMOLYSIS BULLOSA. JT Wright, KI Hall, T Deaton and J-D Fine. Departments of Pediatric Dentistry & Dermatology, Schools of Dent. & Med., The University of North Carolina at Chapel Hill, NC USA.

Although generalized enamel hypoplasia is seen clinically in junctional EB (EBJ) patients, the character of enamel remains poorly defined in the various EB types. While evaluating the oral manifestations of EB, exfoliated or therapeutically extracted teeth were collected for study. Our purpose was to evaluate the structure of different EB enamels. Teeth from individuals with EBDR (n=16), EBDD (n= 2), EBJ (n=6) and EBS (n=4) were examined using light microscopy (LM) and scanning electron microscopy (SEM). The teeth were cut producing sections 100μm thick for LM. The enamel porosity was evaluated using LM and imbibing tooth sections in H_2O or chloronaphthalene. SEM specimens were either fractured or cut, polished and etched with H_3PO_4 for 60 S.

Non-junctional EB enamel was of normal thickness with a typical prismatic structure. However, small hypoplastic enamel pits (≤15μm diameter), not visible clinically, were seen with SEM on the surface of some EBDR teeth. Regions of EBDR enamel also showed an altered crystallite morphology and packing. Altered crystallite habits occurred locally in EBDR enamel and were not observed in all cases. EBJ enamel showed defects of both the prism structure and orientation. In severely affected EBJ teeth the enamel prism course was markedly altered. Teeth from 4 severely affected EBJ (Herlitz) individuals showed only a thin covering (≈40 μm) of prismless enamel. Some areas of the enamel appeared to be missing altogether in these EBJ cases. Other EBJ teeth showed a normal prismatic enamel interrupted by surface pitting. Areas of severe pitting in EBJ enamel often were opaque when viewed with LM. These areas cleared upon imbibition with H_2O or chloronaphthalene indicating the EBJ enamel was porous. EBJ enamel crystallites usually appeared morphologically normal, although, a variety of crystal habits were observed.

Generalized pitting or thin enamel occurred primarily in EBJ cases, however, a variety of localized defects such as altered crystallite morphology and minor surface pitting were seen in other types of EB enamel. Structural evaluation of EB enamel confirms the clinical studies that EBJ enamel is the most severely altered. While there is extreme variation in the histological appearance of enamel in different EBJ cases, this study further indicates that the severity of the enamel defects in EBJ correlates with the severity of the systemic disease.

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THE MINERAL COMPOSITION OF TOOTH ENAMEL IN HEREDITARY EPIDERMOLYSIS BULLOSA. JT Wright, KI Hall, T Deaton and J-D Fine. Depts. of Pediatric Dentistry & Dermatology, Schools of Dent. & Med., The University of North Carolina at Chapel Hill, NC.

Enamel hypoplasia is a common feature of junctional EB (EBJ) while other EB types tend not to have grossly hypoplastic enamel (Gedde-Dahl 1971; Wright et al., 1993). Individuals with recessive dystrophic EB (EBDR) and EBJ are at increased risk for developing dental caries compared with unaffected individuals. The purpose of this study was to examine the enamel mineral content of individuals with EB. Teeth from 19 individuals with EBDR (n=12), dominant dystrophic EB (n=1), EBJ (n=4), EB simplex (n=2) and 10 normal teeth were examined. Thin sections were cut from the methacrylate embedded teeth and particles 50 - 150μg dissected off for analysis. The particle density and volume were established using calibrated density-gradient columns and the particles ashed at 600° C. The Ca and Mg contents were measured by atomic absorption. Colorometric analysis was used for P determination and F was determined using a mini-micro diffusion method.

The mineral content of non-carious EB enamel ranged from 59-100% while normal enamel ranged from 72-99%. Nine individuals with EBDR had a relatively normal enamel mineral content (mean range; 78-83%) while 3 had a slightly decreased enamel mineral content (mean range; 74-77%) compared with normal enamel. There was no statistical relationship between the enamel mineral content and the caries score (Decayed, Missing, Filled Surfaces) ($R = 0.301$, $p = 0.342$) in individuals with EBDR. All 4 EBJ teeth had areas with a decreased enamel mineral content (range; 65-85%). EB simplex enamel showed a mineral content (means; 86%, 79.3%) similar to normal enamel. The Mg content of EB enamel (1450-4500 ppm) also was similar to normal enamel (1415-5000 ppm). While the F content of EB enamel (range; 120-880 ppm) was generally similar to normal enamel (120-540 ppm), there were significant differences between EBJ and other EB enamel types. Interestingly, the highest F content was seen in EBJ enamel (mean range; 490-610 ppm).

We conclude that EBJ enamel is frequently reduced in mineral content. The high F content in EBJ teeth may reflect developmental disturbances during a specific period that result in elevated retention of fluoride during enamel crystallite growth. While this sample size is clearly insufficient for drawing definitive conclusions, a decreased enamel mineral content does not appear to be a major etiologic factor associated with increased dental caries risk in EBDR.

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DNA DIAGNOSIS OF EPIDERMOLYSIS BULLOSA SIMPLEX BY DIRECT AUTO-SEQUENCING: DETECTION OF A MUTATION OF LEU^{122} TO PHE IN K14 OF A SPORADIC CASE OF EBS.

K Yamanishi, M Matsuki, K Konishi, and H Yasuno, Department of Dermatology, Kyoto Prefectural University of Medicine, Kamigyo-ku, Kyoto, Japan.

Epidermolysis bullosa simplex (EBS) has been shown to arise from mutations of the genes for basal keratins K5 and K14. To screen the mutations of the keratin genes, we designed a detection system using PCR and automated sequencing. The sequences in which the reported hot spots of the keratin gene mutations cluster were amplified by PCR using the primers one of which was 5'-phosphorylated. After digestion of the amplified 5'-phosphorylated DNA by λ-exonuclease, the remaining single-stranded DNA was used as a template for dideoxy sequencing with a fluorescent primer. DNA sequences were read by DSQ-1, an automated DNA sequencer. When a case of Köbner type EBS was analyzed, we found a mutation of Leu^{122} to Phe of K14 by a point mutation of C to T transition in one of the alleles. There were no mutations in the members of the patient's family and normal controls. Thus, this case was diagnosed as a sporadic case of EBS by a new mutation of K14 gene with a possible dominant inheritance. Our detection method is useful for screening of the mutations of K14 and K5 genes in EBS and will be applicable to prenatal diagnosis of EBS.

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UNCEIN, A CELL MEMBRANE-ASSOCIATED ANCHORING FILAMENT COMPONENT, IS BIOCHEMICALLY SIMILAR TO KALININ BUT DIFFERS MARKEDLY IN ITS IN VITRO EXPRESSION BY NORMAL AND NON-HERLITZ JUNCTIONAL EPIDERMOLYSIS BULLOSA (JEB) HUMAN KERATINOCYTE MONOLAYERS. Li Zeng, Anthony Daniels, Kathryn Riddelle, and Jo-David Fine, Department of Dermatology, University of North Carolina at Chapel Hill, Chapel Hill, NC.

Uncein, a protein recently defined by the monoclonal antibody (MCAb) 19-DEJ-1, is one of two known components of the anchoring filament (AF). Compared to kalinin (nicein; epiligrin), another AF-associated protein, uncein is undetectable in all junctional epidermolysis bullosa (JEB) skin, suggesting a likely pathophysiological role for an abnormality of uncein in JEB. To better characterize uncein, ^{35}S-methionine-labeled normal (NHKs) and non-Herlitz JEB human keratinocytes (JEBKs) were subjected to immunoprecipitation (IP) with MCAbs to uncein or kalinin, followed by autoradiography. Three distinct protein bands (165, 130, 100 kD) were identified by each antibody. Pre-IP with antibody to one AF protein did not block subsequent IP with the second antibody. Identical protein bands were isolated from spent NHK supernatant via affinity chromatography with purified anti-uncein MCAb. When NHKs and JEBKs were grown as monolayers and examined by indirect immunofluorescence, kalinin was noted in broad, dense, uniform array exclusively between cells. No differences were seen in kalinin expression in these two cell types. In contrast, on NHKs uncein was present in uniform stippled array on the cell surfaces, as well as in filamentous array within the cytoplasm; the extracellular spaces were conspicuously devoid of uncein staining. In contrast, many JEBKs lacked any visible uncein staining. In other cultured JEBKs, uncein was present intracytoplasmically in focal, apical, arciform deposits. Following mechanical removal of the NHKs and JEBKs, bright kalinin staining was seen underneath and between cells. Uncein was noted in only trace, focal, stippled deposits underneath NHKs and was undetectable underneath JEBKs. Whereas essentially equally amounts of uncein and kalinin were detected by IP in radiolabeled spent NHK supernatant, much greater amounts of kalinin were extractable from the petri dish surfaces following dissolution of cells. Kalinin but not uncein was detectable in JEBK supernatant. Taken in context with differences in the site of binding on AFs by each MCAb, our findings suggest that while uncein and kalinin may share biochemical similarities, uncein is closely associated with keratinocyte cell membranes whereas kalinin is truly an extracellular matrix protein, and that the in vitro expression of uncein is markedly abnormal in non-Herlitz JEBKs.

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TWO POLYCLONAL ANTIBODIES TO THE ANCHORING FILAMENT PROTEIN, UNCEIN, REVEAL DIFFERENCES IN SUBUNIT RECOGNITION AND IN TISSUE AND ULTRASTRUCTURAL LOCALIZATION. Li Zeng, Anthony Daniels, Kathryn Riddelle, Donna Cronce, Robert Briggaman, and Jo-David Fine, Dept. of Dermatology, University of North Carolina at Chapel Hill, Chapel Hill, NC.

Uncein, a component of the anchoring filament (AF) originally defined by the monoclonal antibody (MCAb) 19-DEJ-1, is composed of three distinct subunits (165, 130, & 100 kD). Silver enhanced immunoelectron microscopy (IEM) with 19-DEJ-1 has confirmed immunofluorescence (IF) findings of MCAb binding restricted to the dermoepidermal junction (DEJ) and to the epidermal portion of NaCl-split skin basement membrane (BM). Of importance, IF on junctional EB (JEB) skin with 19-DEJ-1 has failed to reveal any detectable immunoreactivity. Unfortunately, 19-DEJ-1 does not immunoblot by conventional technique, thereby limiting its utility as a probe for screening normal and JEB keratinocyte cDNA libraries. To circumvent this problem, eluates of normal human keratinocyte (NHK) supernatants passaged through a 19-DEJ-1 affinity column were used as immunogens for the production of two BM-specific rabbit anti-human polyclonal antibodies (PCAbs), Li-1 & Li-2. Surprisingly, markedly different antibody binding profiles were observed. Li-1 stained all skin BMs (DEJ; vascular; appendageal), identical to antibodies directed against more ubiquitous BM proteins. Although detectable by IEM on both sides of NaCl-split skin, Li-1 decorated AFs predominantly along the lowermost portion of the lamina lucida, reminiscent of kalinin. Li-1 immunoprecipitated (IP'd) a 165 kD protein from NHK supernatant and lacked evidence by dot blot of cross-reactivity with intact human laminin. In contrast, Li-2 bound solely along the DEJ. IEM demonstrated Li-2 binding to AFs along both halves of NaCl-split skin BM, although the predominant localization was to the epidermal portion, most similar to the findings observed with 19-DEJ-1. Li-2 IP'd and immunoblotted 165 and 130 kD protein bands from NHK supernatants. Whereas Li-1 stained all JEB skin specimens normally, reduced or absent staining was seen in about half with Li-2. Although both PCAbs appear to bind to one or more subunits of uncein, the disparate findings by IF, IEM, and IP suggest that different epitopes are being recognized, and that some (particularly via Li-1) may be cross-reactive with kalinin. Furthermore, the staining of all BMs in normal human skin with Li-1 suggests that uncein may be present in all skin BMs but is seen only along the DEJ by 19-DEJ-1 and Li-2, due to epitope masking, or alternatively that uncein may share one or more antigenic domains with other more ubiquitous BM proteins.

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ASSESSMENT OF THE CUTANEOUS BASEMENT MEMBRANE IN SULFUR MUSTARD-INDUCED TOXICITY. Z Zhang, BP Peters, and NA Monteiro-Riviere. Cutaneous Pharmacology and Toxicology Center, North Carolina State University, Raleigh, NC, USA.

The basement membrane (BM) components facilitate epidermal-dermal interaction, but also control cell behavior. The purpose of this study was to examine the effects of bis-2-chloroethyl sulfide (sulfur mustard, HD) on the BM in the process of vesication caused by HD. This was accomplished by (1) examination of the BM components for direct modification by HD, and (2) evaluation of cell proliferation and adhesive activity on HD-treated BM. Normal human foreskin epidermal keratinocytes (NHEK) were biosynthetically labeled with ^{35}S-cysteine. ^{35}S-labeled fibronectin, heparan sulfate proteoglycan, and laminin were immunoprecipitated from the cell culture medium. These immunoprecipitates were treated with HD or ethanol as control, then analyzed by reduced sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). On reduced SDS gels, these three BM components not treated with HD showed the typical profile of dissociated subunits. However, HD treatment caused the appearance of higher molecular weight bands indicative of cross-linking of subunits within these BM molecules. Finally, the extracellular matrices (ECM) synthesized by NHEKs were treated with HD or ethanol, and human keratinocytes were replated on the matrices for 72 hrs. Cell adhesion assay was significantly decrease on HD-treated matrix, indicating that HD-treated ECM loses its ability to promote cell adhesion and proliferation. These findings demonstrate a potential role of HD-alkylated BM in dermal injury caused by HD. **(SUPPORTED BY USAMRDC, DAMD17-92-C-2071)**

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PRENATAL DIAGNOSIS OF EPIDERMOLYSIS BULLOSA

I. Anton-Lamprecht, Department of Dermatology, University of Heidelberg, Heidelberg/Germany

At the time of the EB symposium a total of 200 cases of fetal skin sampling for prenatal diagnosis had been investigated in Heidelberg, among them 106 for risk of EB (32 affected). 83 of these cases were at risk of junctional EB (80 Herlitz, 27 affected, 7 with pyloric obstruction, 2 affected) and 19 at risk of dystrophic EB (18 Hallopeau-Siemens, 4 affected, 1 affected with EBD Cockayne-Touraine). 4 further fetuses at risk of undetermined EB types were unaffected. Our series includes 2 twin pregnancies with both siblings healthy in either pregnancy. Prenatal diagnosis was performed mainly in cooperation with the gynecologists Professor Rauskolb, Northeim/FRG, Docent Dr. Gustavii, Lund/Sweden, and Professor Holzgreve, Münster/FRG. Fetal skin sampling is done under real ultrasound guidance using small cannulas and forceps of about 1.2 mm diameter. The time of fetal skin sampling regularly was between week 18 and 20, now changed to earlier times in the second trimester (week 16 to 18). In our experience prenatal diagnosis of EB is quite safe and absolutely reliable with no risk for false-negative or false-positive diagnostics provided a good quality of fetal skin samples.

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EXPERIENCES WITH LARGE STUDY COHORTS: INVESTIGATION OF EB IN HEIDELBERG

I. Anton-Lamprecht, Department of Dermatology, University of Heidelberg, Heidelberg/Germany

We are dealing with EB in Heidelberg since 1971, when we started with our systematic clinical, genetic, and ultrastructural investigations (cooperation: U.W.Schnyder, I.Hashimoto, T.Gedde-Dahl). Patients in this study came from all over Germany, Scandinavia (including Gedde-Dahl's Norwegian patients) and many other European and extra-European countries, including Japan and Australia.

Our investigations led to the distinction of the 3 major EB groups based on their plane of separation and basic structural abnormalities. This biologically based Heidelberg EB classification, first proposed in 1977, is now internationally accepted and more or less identical to the US classification based on data from the National EB Registry. It reflects the differences of the basic abnormalities, that are just now being confirmed as causative with the identification of mutations concerning basal cell keratins (**EB simplex group**), hemidesmosomes (**EB atrophicans group**) and anchoring fibrils/collagen VII (**EB dystrophica group**).

Until to IV/1994, a total of 599 EB cases have been investigated by EM in Heidelberg. Roughly one third of the cases belongs to each of the three major EB groups. In the **EB simplex group** (160=28,6%), EB herpetiformis Dowling-Meara (76=13,6%) is the most frequent type, followed by the Köbner type (44=7,9%). 3 of the Köbner cases lacked basal keratins entirely by EM. In the Dowling-Meara type, keratin clumps and aggregates normally occur side by side in the same cell and tissue sample and thus are not indicative of different types of mutations as suggested by Kitajima (1993).

In junctional EB (**EB atrophicans group**, 185=33,1%), the Herlitz type is the most frequent one (111=19,9%); it accounts for 42-50% of all EB newborns in our material. 8 cases of junctional EB with pyloric obstruction (1,4%) had the severest degree of hemidesmosome hypoplasia, while patients with non-lethal junctional EB (mitis type, 32=5,7%) can be identified by their much better hemidesmosomes but lack mature subbasal dense plates. Various types of hypoplastic hemidesmosomes indicate mutations of different constituents of hemidesmosomes.

In the **EB dystrophica group** (214=38,3%), the recessive Hallopeau-Siemens type with its large spectrum of different severities is the most frequent one (162=29,0%); it accounts for about 27-30% of all EB newborns in our material. Different degrees of defective anchoring fibrils are presented that grossly correlate to the clinical severity. However, while the majority of mutilating EBD cases lack anchoring fibrils entirely, exceptional cases are shown to form large amounts of normal appearing anchoring fibrils that are destroyed secondarily during blister formation.

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THERAPY OF EPIDERMOLYSIS BULLOSA: MANAGEMENT OF ESOPHAGEAL STENOSIS

I. Anton-Lamprecht, Department of Dermatology, University of Heidelberg, Heidelberg/Germany

Surgical replacement of the esophagus by colonic interposition is still widely considered to be the therapy of choice in cases of esophageal obstruction. During the round table discussion of therapeutic options, the Heidelberg experiences with the conservative management of esophageal stenosis in dystrophic EB by means of dilatation with balloon catheters under X-ray control and local or general anesthesia followed by postoperative short- or long-term tube feeding instead of total surgical replacement were presented (cooperation: Prof. Feurle, Neuwied; Prof. Weidauer, Heidelberg; Prof. Seitz, Heidelberg). These experiences include one patient with inverse recessive dystrophic EB. One further patient with mutilating dystrophic EB had a complete obstruction for more than 3 months and after eventual dilatation is now free of complaints for more than 10 years. No incidents such as perforation occurred. Thus, by balloon dilatation of esophageal stenosis, highly burdening major surgery can be avoided.

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MOLECULAR ANALYSIS OF THE REGIONS OF THE COL7A1 GENE CODING FOR THE NC-1 AND NC-2 DOMAINS OF COLLAGEN VII IN PATIENTS WITH RECESSIVE EPIDERMOLYSIS BULLOSA DYSTROPHICA (REBD): A PATIENT WITH REBD MUTILANS IS A COMPOUND HETEROZYGOTE CARRYING TWO MUTATIONS

D.U. Kalinke*, U. Kalinke+, M.Zimmermann*, I.Anton-Lamprecht$, J.O.Winberg£, A.M.Christiano", J. Uitto" and L. Bruckner-Tuderman**Dept.of Dermatology* and Inst.f.Exper.Immunology+, University Hospital, Zürich, Switzerland. Dept.of Dermatology, University of Heidelberg $, Germany. Polar Inst. for Medical Genetics £, Tromsö, Norway. Dept.of Dermatology, Jefferson Medical College", Philadelphia, PA, USA. Dept.of Dermatology, University of Münster **, Germany.

In the skin of patients with the mutilating type of REBD anchoring fibrils are rudimentary or absent as determined by electron-microscopy, leading to severe sub-lamina densa blistering of the skin. Collagen VII is the major component of the anchoring fibrils. It's gene, COL7A1, is the candidate gene linked to REBD. Immunofluorescence staining of fibroblasts from 7 patients with REBD mutilans with antibodies to collagen VII was faint or negative. However, Northern blot analysis revealed normal size mRNA in all patients fibroblasts. Overlapping fragments, corresponding to the coding sequence of the collagen VII gene (COL7A1), were amplified with RT-PCR and analyzed with PCR-SSCP. In one R-EBD mutilans-patient a 25 bp deletion was detected in the gene region coding for the NC-1 domain, inherited by his healthy father, leading to a premature stop codon. In the same patient a second mutation was found which creates a leucine to proline substitution in the NC-2 domain. As determined by PCR-SSCP analysis both parents have no band shifts in the NC-2 domain. The REBD patient represents most likely a compound heterozygote with a premature stop codon on one allele and a leu to pro substitution on the other allele.